Preconditioning with acute and chronic lithium administration reduces ischemia/reperfusion injury mediated by cyclooxygenase not nitric oxide synthase pathway in isolated rat heart

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ARTICLE INFO

Article history:
Received 11 March 2008
Accepted 20 August 2008
Available online 22 August 2008

Keywords:
Lithium
Ischemia/reperfusion
Rat heart
Nitric oxide synthase
Cyclooxygenase

ABSTRACT

Lithium is widely used for the management of neuropsychiatric symptoms in bipolar disorders. A variety of hypotheses have been invoked to explain the mechanism of action of lithium. To determine if lithium exerts direct cardioprotection, in the present study perfused rat heart model was used. The mechanism of lithium-mediated cardioprotection was explored by combined use of lithium and nitro-l-arginine methyl ester (L-NAME, a non-selective nitric oxide synthase inhibitor) or indomethacin (a non-selective cyclooxygenase pathway inhibitor). Rat isolated hearts were used for Langendorff perfusion. Hearts were either non-preconditioned or preconditioned with acute lithium (3 mM) or chronic lithium (600 mg/l in tap water for 4 weeks, 0.265±0.023 mM in serum) before 30 min global ischemia followed by 90 min reperfusion. Within each of these protocols, hearts were divided into two groups; one group was exposed to L-NAME (0.1 mM) and another group was exposed to indomethacin (10 µM). Infarct size was measured by the triphenyltetrazolium chloride method. Left ventricular function was assessed by left ventricular developed pressure (LVDP), heart rate and coronary flow (CF). In our experiment acute and/or chronic administration of lithium before prolonged ischemia offered significant myoprotective effects in terms of infarct size reduction and improved cardiac function against ischemia/reperfusion injury. The effects of lithium pretreatment were prevented by the administration of indomethacin but not L-NAME. In conclusion, our results demonstrate that preconditioning with acute and/or chronic lithium administration improves recovery of the ventricular function and reduces infarct size via cyclooxygenase (COX) pathway in isolated rat heart.

1. Introduction

Ischemic injury to vital organs such as the heart contributes significantly to morbidity and mortality throughout the world. Deprived of oxygen-carrying blood, cellular respiration slows down with irreversible damage occurring within minutes in tissues such as myocardium. Various methods of limiting ischemia/reperfusion (IR) injury have been described.

It is now accepted universally that a small amount of stress by repeated ischemia and reperfusion can delay the onset of further irreversible injury and reduce the subsequent postischemic ventricular dysfunction in hearts. Such preconditioning effects can be simulated by pretreating the hearts with pharmacological manipulations (Tosaki et al., 1998). Although there is no known drug that completely prevents myocyte necrosis, some agents can slow the rate of cell death.

Lithium, a monovalent cation, has been used therapeutically for more than 50 years and remains an important pharmacotherapeutic agent for treatment of bipolar disorder. Although the mechanisms underlying lithium actions remain unclear, there is increasing evidence that lithium exerts its therapeutic effects by interfering with signal transduction through G-protein-coupled pathways (Jope and Williams, 1994) or direct inhibition of specific targets in signaling system, which include inositol monophosphatase (Nahorski et al., 1991) and glycogen synthase kinase-3 (Lenox and Wang, 2003).

Tong et al. (2002) reported that the clinical use of lithium in the treatment of bipolar disorder is associated with an extremely narrow therapeutic range. Besides neurologic and other side effects, the lithium ion has been shown to produce a variety of cardiovascular effects in man and experimental animals.

Lithium interacts with physiological responses mediated by adenylyl cyclase (Ebstein et al., 1980), nitric oxide (NO) (Nejadkey et al., 2006) and COX products (Paoletti et al., 1998).

A growing body of evidence supports the cardioprotective role of NO. For example, a NO donor or a precursor for NO synthesis like l-arginine has been found to ameliorate myocardial ischemic-reperfusion injury (Xi et al., 1999).

Prostanoids are another system that exerts significant effects on myocyte response to ischemic-reperfusion injury. Prostaglandins have been reported to be endogenous mediators of the protection afforded by ischemic preconditioning (IP) (Arad et al., 1996). Some investigators have demonstrated that acetyl salicylic acid (aspirin; a non-selective
COX inhibitor) could abolish the antiarrhythmic effect of ischemic preconditioning against reperfusion tachyarrhythmias in isolated rat hearts (Arad et al., 1996).

On the other hand, some authors have suggested that nitric oxide or COX products may mediate some of the lithium-induced responses in the brain or peripheral tissues (Bagetta et al., 1993; Nejadkey et al., 2006). Therefore, one may expect lithium to have cardioprotective effects perhaps due to elevation NO and/or COX products. So the inhibition of the nitric oxide synthesis by L-NAME and cyclooxygenase pathway by indomethacin would be expected to decrease lithium-induced preconditioning on the hearts.

The aims of the present study were, therefore, firstly to examine the preconditioning effects of acute and/or chronic lithium administration on ischemic reperfused hearts, and secondly, the role of cyclooxygenases and nitric oxide synthase pathways in these cardioprotective effects were evaluated.

Most studies addressing the mechanism of preconditioning (PC) to date have used infarct size as their end point and very few have been concerned with mechanical function. We assessed the preconditioning effect of lithium on both mechanical function and infarct size in the isolated rat heart.

2. Materials and methods

2.1. Animals

Experiments were performed in accordance with the recommendations of the Ethics Committee on Animal Experiments of the Medical School (Tehran University of Medical Sciences, Tehran, Iran). Male Sprague-Dawley rats weighing 250–300 g were used in this study. All animals were given free access to food and water. Thoracotomy was performed under anesthesia induced by an intraperitoneal (i.p.) injection of 60 mg/kg pentobarbital sodium 30 min after treatment with heparin (500 IU).

2.2. Langendorff perfusion

The aorta was cannulated and the heart was retrogradely perfused in situ to avoid ischemia. Hearts were then excised and mounted on a non-recirculating, constant-pressure (80 mm Hg) Langendorff perfusion system, and were perfused with an oxygenated (95% O₂ – 5% CO₂) normothermic (37 °C) Krebs’–Henseleit bicarbonate (KHB) buffer which had the following composition (in mM): NaHCO₃ 25; KCl 4.7; NaCl 118.5; MgSO₄ 1.2; KH₂PO₄ 1.2; glucose 11; CaCl₂ 2.5 (pH 7.4). The perfusion apparatus was water-jacketed to maintain a constant perfusion temperature of 37 °C and, during prolonged global ischemic periods, hearts were immersed in KHB buffer at 37 °C. Hearts were allowed to beat spontaneously throughout the experiments. A latex, fluid-filled, isovolumic balloon was introduced into the left ventricle through the left atrial appendage and inflated to give a preload of 8 to 10 mm Hg and connected to a pressure transducer (Harvard). Hemodynamic data were monitored with a home-made program. Left ventricular developed pressure (LVEDP), heart rate (HR), and coronary flow (CF) were registered at regular intervals. Ischemia was achieved by clamping the aortic perfusion catheter in such a way that coronary flow was reduced to zero. Our Langendorff apparatus permits instantaneous change of the perfusion fluids from standard KHB to one containing different pharmacological solutions by adjusting an inlet valve to the aortic perfusion cannula. Developed pressure is defined as the peak of systolic minus end-diastolic pressure. Left ventricular function was assessed by left ventricular developed pressure, the Rate Pressure Product (RPP=heart rate×left ventricular developed pressure) and coronary flow.

2.3. Experimental protocol

The experimental protocol used is shown in Fig. 1. Hearts were perfused for 25 min to establish equilibrium hemodynamics. Equilibrium phase was terminated when left ventricular developed pressure, heart rate and coronary flow were maintained at the same level for three continuous periods of measurement timed 5 min apart. Baseline measurements were recorded at the end of this time. Hearts not meeting these criteria were not used in the study. After 25 min of perfusion, hearts were divided into 12 groups: control hearts (control) were perfused without global ischemia at 37 °C for 165 min; global ischemia hearts (GI) were subjected to 30 min ischemia and 90 min reperfusion; ischemic preconditioned hearts (IP) were subjected to 5 min of zero-flow global ischemia followed by 5 min reperfusion with Krebs–Henseleit buffer; before 30 min of global ischemia; acute lithium hearts (A-Li) were perfused with 0.5, 1, 3 and 5 mM lithium for 10 min before global ischemia; L-NAME plus acute lithium hearts (A-Li+L-NAME) were perfused with 0.1 mM L-NAME+3 mM lithium for 10 min before global ischemia; indomethacin plus acute lithium hearts (A-Li+Indo) were perfused with 10 μM indomethacin+3 mM lithium for 10 min before global ischemia; chronic lithium hearts (C-Li), pretreated with lithium chloride in tap water for a period of 4 weeks, were subjected to 30 min ischemia and 90 min reperfusion; L-NAME plus chronic lithium hearts (C-Li+L-NAME) were perfused with 0.1 mM L-NAME for 10 min before global ischemia; indomethacin plus chronic lithium hearts (C-Li+Indo) were perfused with 10 μM indomethacin.

Fig. 1. Schematic illustration of experimental groups. GI, global ischemia; IP, ischemic preconditioning; Li, acute lithium; L-NAME, nitro-L-arginine methyl ester; Indo, indomethacin.
for 10 min before global ischemia. The numbers of animals in each group were 7 rats.

The concentration of L-NAME (Taylor et al., 2007) and indomethacin (Bouchard et al., 2000) were derived from the previous studies. The concentration of acute lithium was selected following preliminary dose–response study for lithium chloride, whereas the dosage of chronic lithium was derived from the previous study (Dehpour et al., 2000). Animals received 600 mg/l lithium chloride in tap water for a period of 4 weeks. At the end of the treatment period, serum lithium levels were determined by an atomic absorption spectrophotometer. Left ventricular developed pressure, heart rate and rate pressure product were expressed as percentages of the baseline values in the experiments.

2.4. Measurement of infarct size

At the end of the reperfusion period, hearts were frozen and kept in a −20 °C freezer to facilitate slicing of 2 mm transverse sections across the long axis. All hearts had approximately the same size (1.2 cm; atria and great vessels excluded). Slices were incubated in 1% triphenyltetrazolium chloride (TTC) in a phosphate buffer (pH 7.4) for 30 min at 37 °C. After staining, slices were immersed in 10% formalin to enhance the contrast between stained and unstained tissues. Tissues that were stained brick red were taken as viable, whereas pale or white tissues were taken as necrotic. The areas of the left ventricle and infarcted tissues were measured by way of a planimetry from the scanned hearts by using Photoshop. 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.

2.5. Chemicals

Indomethacin, L-NAME and triphenyltetrazolium chloride were obtained from Sigma-Aldrich (Deisinhofen, Germany). Lithium chloride and general laboratory chemicals were acquired from Merk (Darmstadt, Germany). Indomethacin, L-NAME and lithium chloride were dissolved in Krebs’–Henseleit bicarbonate buffer.
2.6. Statistical analysis

Computerised (SPSS 11.5) one-way analysis of variance (ANOVA), followed by the Tukey test was used to determine the statistical significant differences. Data are expressed as the mean±S.E.M. Differences were considered statistically significant when *P* was less than 0.05.

3. Results

3.1. Base line and cardiac function during interventions

No significant differences were observed between or within groups after 25 min of stabilization period for the parameters examined (left ventricular developed pressure, heart rate and coronary flow).

3.2. Postischemic functional recovery

Postischemic left ventricular function was assessed by left ventricular developed pressure, heart rate, rate pressure product and coronary flow. After onset of ischemia, left ventricular developed pressure declined to zero within 5 min and remained at this level during ischemic period in all groups. No left ventricular developed pressure was detected at the end of 30 min of ischemia. Because heart rate and left ventricular developed pressure recover to different degrees, in the present study rate pressure product is presented as a reliable index of contractile function. With the onset of global ischemia, rate pressure product fell to zero within 5 min and remained at this level during the ischemic period in all groups. Upon reperfusion, the rate pressure product displayed a gradual recovery throughout reperfusion.

Lithium improved the rate pressure product in a concentration-dependent manner (Fig. 2). In the acute lithium hearts, 0.5, 1, 3 and 5 mM of lithium, rate pressure product ultimately recovered to 73±3.8%, 80±4%, 86±3.8% and 61±3.3% of pre-ischemic levels, respectively. The effective concentration of lithium for recovery of the rate pressure product was 3 mM, so we used that to induce preconditioning.

Rate pressure product of different groups is shown in Figs. 3 and 4.

In the control and global ischemic hearts and the hearts preconditioned with ischemia, acute lithium, L-NAME+acute lithium, indomethacin+acute lithium, chronic lithium, L-NAME+chronic lithium

![Fig. 4. Mean of the recovery of RPP (Basal Value %) in A-Li, C-Li, A-Li+L-NAME, A-Li+Indo, C-Li+L-NAME, C-Li+Indo groups. (n=7). A-Li, acute lithium; C-Li, chronic lithium, Indo, indomethacin; L-NAME, nitro-ω-arginine methyl ester. *Significant difference with A-Li group (*P*<0.05). &Significant difference with C-Li group (*P*<0.05).](image)

![Fig. 5. Infarct size (% of left ventricular) in Li (0, 0.5, 1, 3, 5 mM) groups. (n=7). Li, acute lithium.](image)
and indomethacin+chronic lithium, the rate pressure product ultimately recovered to 98±2.8%, 57±4.7%, 92±3.2%, 86±3.8%, 83±1.5%, 62±4%, 80±2.6%, 76±2.5% and 34±3.9% of pre-ischemic levels, respectively. Preconditioning with both acute and chronic lithium improved the posts ischemic cardiac function and increased the recovery of rate pressure product (P<0.05 vs global ischemic hearts). There were no significant differences in the rate pressure product in both acute and chronic lithium in comparison to the ischemic preconditioning group. Blockade of COX by indomethacin significantly depressed posts ischemic recovery of the rate pressure product in acute lithium+indomethacin and chronic lithium+indomethacin groups (P<0.05 vs acute and chronic lithium groups). L-NAME was not able to block the recovery of the rate pressure product produced by acute and chronic lithium.

Coronary flow fell to zero during ischemia and, at the start of reperfusion recovered to between 95 and 100% of pre-ischemic levels in all groups.

3.3. Infarct size

There were no significant differences in the left ventricular area between the groups. Infarct size of different groups is summarized in Figs. 5 and 6. Infarct size in the control group was 0.40±0.16% and this was significantly increased in the global ischemia group (31±1.2%, P<0.05). Preconditioning with both acute and chronic lithium significantly protected the ischemic myocardium, and reduced infarct size to 15±1.9% and 13±2.8% respectively vs 31±1.2% in the global ischemia group (P<0.05). As shown in Fig. 6, lithium decreased infarct size in a concentration-dependent manner. Maximum effect was induced by 3 mM of lithium chloride. Treatment with indomethacin significantly abolished the effect of acute and chronic lithium in reducing infarct size, resulting in an increased infarct size in acute lithium+indomethacin and chronic lithium+indomethacin groups to 24±3.3% and 27±3% vs 15±1.9% and 13±2.8% in the acute and chronic lithium groups respectively (P<0.05). L-NAME was not able to block the reduction of infarct size induced by acute and chronic lithium (19±3.3% and 17±4.1% in the acute lithium+L-NAME and chronic lithium+L-NAME groups respectively).

4. Discussion

The results of this study demonstrate that pretreatment of the rat hearts with acute and/or chronic lithium before ischemia/reperfusion, significantly improved the recovery of posts ischemic ventricular function and reduced the infarct size compared to that seen in un preconditioned hearts. The evidence in this study indicates that lithium plays a fundamental biological role in protecting the hearts against ischemia/reperfusion injury. The cardioprotective effect of lithium was blocked by a non-selective COX inhibitor, indicating an essential role of COX pathway in lithium-induced cardioprotection. Whereas inhibition of NOS by a non-selective NOS inhibitor was ineffective, which suggests that the effect of lithium in the isolated rat heart is not mediated by the NOS pathway.

There are several salient findings of the present study which indicate that lithium may act as a pharmacological preconditioning agent. The most important finding, is that acute lithium in a dose-dependent manner could precondition the heart, as evidenced by its ability to lower the infarct size and to improve postischemic functional recovery. However, another study has reported different data, which show that lithium had no direct effect on cardiac function in the isolated, perfused rat heart (Linakis et al., 2000). We tried four different doses of lithium (0.5, 1, 3 and 5 mM) and among them 3 mM lithium was found to be optimal. In our experiment chronic administration of lithium before prolonged ischemia offered myoprotective effects in terms of infarct size reduction and improved cardiac function against ischemia/reperfusion injury. The results suggest that these effects of chronic lithium occur at concentrations (0.265±0.023 mM) below the therapeutic levels in patients, which are usually 0.8–1.0 mM (Gelenberg et al., 1989). The benefits of lithium pretreatment were prevented by the administration of indomethacin, indicating that lithium-induced protection was mediated by the cyclooxygenases pathway. Although lithium has been used for over 50 years to treat bipolar disorder and has been suggested to be neuroprotective (Chen and Chuang, 1999), the basis of its therapeutic effect remains unclear. The arachidonic acid cascade, which plays a key role in brain signaling (Axelrod, 1990) could represent a target of lithium and other mood-stabilizers. Cyclooxygenase catalyzes the oxidation and metabolism of arachidonic acid, leading to the formation of prostanooids. Arachidonic acid is released in response to receptor activation and pathological stimuli such as myocardial ischemia. It has been demonstrated that release of arachidonic acid elicits a positive cardiac inotropic response (Damron and Summers, 1997). Paletti et al. (1998) found that administration of lithium and tacrine (an anti cholinesterase agent) enhance COX-2 immunoreactivity and elevates brain PGE2 content,
suggesting that lithium and tacrine increase the COX enzyme activity. The latter concept is supported by the evidence that, treatment with indomethacin, an inhibitor of COX activity, not only minimized basal production of PGE2, but also abolished the elevation evoked by lithium and tacrine (Paoletti et al., 1998). Therefore, one may expect that cardioprotection induced by lithium may be due to an elevation in the content of COX metabolites in the hearts. So, we have tested, using indomethacin, whether prostaglandins are involved in the protection afforded by lithium. Our results suggest that endogenously produced cyclooxygenase metabolites play a role in the heart protection afforded by lithium. To our knowledge, this is the first study to implicate lithium as a pharmacologic preconditioning agent. This is also the first study to examine the role of COX pathway in the lithium-induced cardioprotection in the isolated rat heart model.

Although the use of COX inhibitors is common, the effect of COX inhibition on heart, particularly during ischemia and reperfusion, is not well understood. Inhibitors of COX have been reported to enhance injury in many tissues (Adderley and Fitzgerald, 1999). Michael et al. reported that COX-2 is important for the function of the heart during ischemia/reperfusion, because hearts from COX-2−/− mice showed a significant decrease in postischemic recovery of left ventricular developed pressure (Camitta et al., 2001). These data suggest caution in the use of COX inhibitors in patients with ischemic heart disease. The pathophysiological role of COX-2 is much more complex. This enzyme may exert either beneficial or deleterious effects depending on the intensity of its induction, the pathophysiological setting, and the activity of specific cells to metabolize PGH2 produced by COX-2 into cytoprotective prostanooids. Our results indicate that COX is a critical component of lithium-induced protection in the isolated rat heart. The role of cyclooxygenase enzymes as obligatory mediators of myocardial protection produced by diverse preconditioning stimuli may have implications for the clinical use of COX inhibitors.

In vitro studies indicate that lithium can induce renal medulary interstitial cell cyclooxygenase 2 (COX2) protein expression via inhibition of glycogen synthase kinase-3β (GSK-3β) (Rao et al., 2005). Lithium is known to be a GSK-3β inhibitor in the heart and inhibition of GSK-3β is cardioprotective (Tong et al., 2002). Taken together, these data and our results suggest that COX signaling acts as one modulator for GSK-3β linked preconditioning.

In our experiment L-NAME did not reduce the cardioprotective effects of acute and/or chronic lithium. These results suggest that NO is not involved in the mechanism of lithium-induced protection in the isolated rat heart model.

The majority of the studies published in the past decade support a cytoprotective role of NO in myocardial ischemia/reper fusion injury, both in vitro and in vivo (Bolli, 2001). A small fraction of studies have reported that inhibition of NOS was found to have no discernible effect on ischemia/reperfusion injury and to be detrimental in some studies (Bolli, 2001). Considering the numerous differences among investigations with respect to experimental protocols, dosages of drugs, etc., a certain degree of variance in outcomes should be expected. Yao and Gross (1993) and Bugge and Ytrehus (1996) found that acetylecine-induced and/or bradykinin-induced preconditioning, were not blocked by NOS inhibitors in open-chest dogs (Yao and Gross, 1993) and isolated rat heart (Bugge and Ytrehus, 1996) respectively. In contrast, Richard et al. (1995) reported that acetylecine-induced early preconditioning was blocked by L-NA in open-chest rats.

Previous studies from our laboratory and by others have demonstrated that the administration of lithium could increase NOS activity (Dehpour et al., 2000; Nejadkey et al., 2006). Pretreatment of rats with L-NAME reduced the protective effect of lithium against ethanol-induced gastric damage (Nejadkey et al., 2006). Furthermore, it has been reported that lithium generates NO by increasing the NOS activity in the rat hippocampus (Bagetta et al., 1993). These results suggest that NO may be involved in the effects of lithium in other tissues, but not in cardiac protection induced by lithium in this model.

However, it is not yet clear whether these disparities are due to the differences between species or in the methods used, and more detailed studies are needed to verify the exact mechanism of action of lithium on NOS activity in this tissue.

Finally, our results demonstrated that the treatment with L-NAME for 10 min before ischemia, however, did not alter recovery of cardiac contractility and reduction of infarct size from ischemic preconditioning, while inhibition of COX pathway was detrimental. It seems that protection induced by lithium is not mediated by the generation of NO but is mediated by the generation of COX metabolites during preconditioning with lithium.

Acknowledgments

We would like to acknowledge the Tehran University of Medical Sciences for financial support of this research and special thanks to professor Farokh Shadan.

References


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