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Research Article
Endogenous Nociceptin Contributes to Remote Aortic Preconditioning via Opioid Receptor Like$_1$ Receptor (ORL$_1$) in Rat Heart

Manisha Lavania and Harlokesh Narayan Yadav

Institute of Pharmaceutical Research, GLA University (Mathura)17KM stone, NH-2, Mathura-Delhi Road, P.O.: Chaumuhan, 281406 Mathura, Uttar Pradesh, India

Abstract

Background and Objective: There are three classic opioid receptors, belong to G-protein super family. Opioid receptor like$_1$ receptor (ORL$_1$) is a newly introduced orphan receptor of this category having highly selective endogenous ligand nociceptin. The ORL$_1$ receptors are well known for their neurobehavioral effects. Recently they are suggested to be involved in prevention of myocardial infarction and prevention of ischemia reperfusion injury. This study is aimed to examine possible involvement of ORL$_1$ receptor in remote aortic preconditioning (RAPC) induced cardio protection in rat heart. **Materials and Methods:** The study has been divided into seven groups (n = 6). Animals (Wistar Albino rats) were either kept untreated or pretreated with vehicle (20% DMSO/water solution)/JTC-801 (ORL$_1$ receptor antagonist; 30 min prior to RAPC induction)/glibenclamide (K_{ATP} channel blocker; 60 min prior to RAPC induction)/co-administration of JTC-801 and glibenclamide. Four repeated episodes comprising of 5 min of abdominal aorta occlusion and 5 min of reperfusion were given to produce RAPC. Thirty minutes of ischemia episode was given to produce myocardial injury followed by 120 min of repercussion. Resultant myocardial injury and myocardial infarct size were measured and compared among the groups. **Results:** The RAPC produced significant reduction in ischemia reperfusion injury ($p<0.001$) in terms of myocardial infarction, lactate dehydrogenate (LDH) and creatine kinase-MB (CK-MB) release. The JTC-801 attenuated the cardioprotective effect of RAPC. Glibenclamide also attenuated the cardioprotective effect of RAPC but combination of both drugs could not produce an additional effect on attenuation of cardioprotection produced by RAPC. **Conclusion:** This study results that endogenous nociceptin, through acting on ORL$_1$ receptor is involved in remote aortic preconditioning induced cardioprotection.

Key words: Remote ischemia preconditioning, remote aortic preconditioning (RAPC), opioid receptor like$_1$ receptor (ORL$_1$), KATP channels, nociceptin

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Corresponding Author: Manisha Lavania , Institute of Pharmaceutical Research, GLA University (Mathura)17KM stone, NH-2, Mathura-Delhi Road, P.O.: Chaumuhan, 281406 Mathura, Uttar Pradesh, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Short episodes of ischemia and reperfusion to distant organ other than heart like renal artery[1-5], abdominal aorta[6] and mesenteric artery[7] have been documented to prevent myocardium against ischemia/reperfusion injury (I/R injury), this phenomenon is termed as remote ischemia preconditioning (RIPC)[8,9]. Although, remote ischemia preconditioning is clinically feasible procedure[10], the mechanisms and signaling pathways underlying this phenomenon of cardioprotection from I/R injury still remain to elucidate. The RIPC stimulus presumably induces release of biochemical messengers like endothelial nitric oxide[11], cyclooxigenase-2[12], opioid peptides[13], adenosine[14] and bradykinin[15] which shows cardioprotection by reducing oxidative stress and prevention of mitochondrial functions. In addition, remote ischemia preconditioning involves activation of K_{ATP} channels[16], suppression of proinflammatory gene[11], expression of antioxidant genes, modulation of gene expression[17] and suppression of blood-borne leukocyte activation[18].

Opioid receptors belong to the large super family of seven transmembrane-spanning (7 TM) G-protein-coupled receptors (GPCRs). They are activated both by endogenously produced opioid peptides and by exogenously administered opiate compounds; opioids produce analgesia by decreasing sensitivity for pain perception. There are three classical opioid receptors, δ-opioid receptor (DOP), k-opioid receptor (KOP) and μ-opioid receptor (MOP)[19-21]. An "orphan" receptor having high degree of homology to the "classical" opioid receptors has been termed as opioid receptor $like_1$ (ORL_1) receptor[22]. Nociceptin/orphanin FQ is a newly discovered heptadecapeptide having high affinity towards opioid receptor $like_1$ receptor (ORL_1)[23]. The ORL_1 receptors are widely distributed in the central nervous system, particularly in forebrain, throughout the brainstem in both dorsal and ventral horns of the spinal cord[24]. The ORL_1 receptors are also present in autonomic nervous system enervated to the heart and blood vessels[24-26] and regulate the turnover of neurotransmitter within synapse[27]. It has been reported that activation of ORL_1 receptor decreases heart rate and blood pressure[24,25,28,29]. The transfer of coronary effluent[30] and hydrophobic constituents of coronary effluent[31] from preconditioned rabbit to virgin acceptor hearts has provided cardioprotection in acceptor hearts through opioid receptors. It has been reported that ORL_1 receptor activation opens the K_{ATP} channels by activation of cAMP and cGMP[32,33]. Expression and activity of ORL_1 receptors get increase during ischemia insult[34,35]. Therefore, it would be worthwhile to investigate the possible involvement of remote aortic preconditioning in well known model of abdominal aorta occlusion in rat heart.

MATERIALS AND METHODS

Animals: Age matched Wistar Albino rats (150-300 g) of either sex were employed in the present study. They were fed on standard laboratory chow (Ashirwad industries Ltd., Ropar, India) and had free access to tap water. Animals were provided 12 h of light and 12 h of dark cycle. The experimental protocol used in the present study was approved by Institutional Animal Ethics Committee.

General surgical procedure: Rats were anaesthetized with pentobarbitone sodium (45 mg kg^{-1}, i.p.). About 2 cm long incision was made on the abdomen. Lower portion of abdominal aorta was isolated below the point of origin of renal artery and a silicone suture (numbered 5/0) was used to make a shoelace knot to occlude the abdominal aorta to produce ischemia and knot was untied for reperfusion. Four episodes of ischemia and reperfusion, each comprising of 5 min of occlusion and 5 min of reperfusion were used to produce remote aortic preconditioning.

Isolated perfuse rat heart: Heparin (500 IU, i.p.) (Gland Pharma Ltd, Hydrabaad, India) was administered to the rats 20 min prior sacrificing the animal by stunning. Heart was rapidly excised and immediately mounted on Langendorff's apparatus[36]. Isolated heart was retrogradely perfused at constant pressure of 80 mmHg with Kreb's Henseleit (KH) buffer NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgSO_4.7H_2O$ 1.2 mM, $NaHCO_3$ 25 mM, KH_2PO_4 1.2 mM and $C_6H_{12}O_6$ 11 mM, pH 7.4 maintained at 37°C bubbled with 95% O_2 and 5% CO_2. Flow rate was maintained at 7-9 mL min^{-1} using Hoffman's screw. The heart was enclosed in doubled wall jacket, the temperature of which was maintained by circulating water heated to 37°C. Global ischemia was produced for 30 min by blocking the inflow of Kreb's Henseleit solution. It was followed by reperfusion for 120 min. Coronary effluent was collected just after starting of reperfusion followed by 1 min and 30 min of reperfusion for estimation of LDH release while CK-MB was measured immediately and 5 min of repercussion.

Preparation and administration of drugs: The JTC-801 (1 mg kg^{-1}, i.p.) was dissolved in 20% solution of DMSO (dimethyl sulfoxide) in water[37]. Glibenclamide[12] (1 mg kg^{-1}, i.p.) was dissolved in water.

Study groups and experimental protocol: The present study was conducted on seven groups and each group comprised of six rats. The diagrammatic representation of experimental protocol (Fig. 1) is given below.

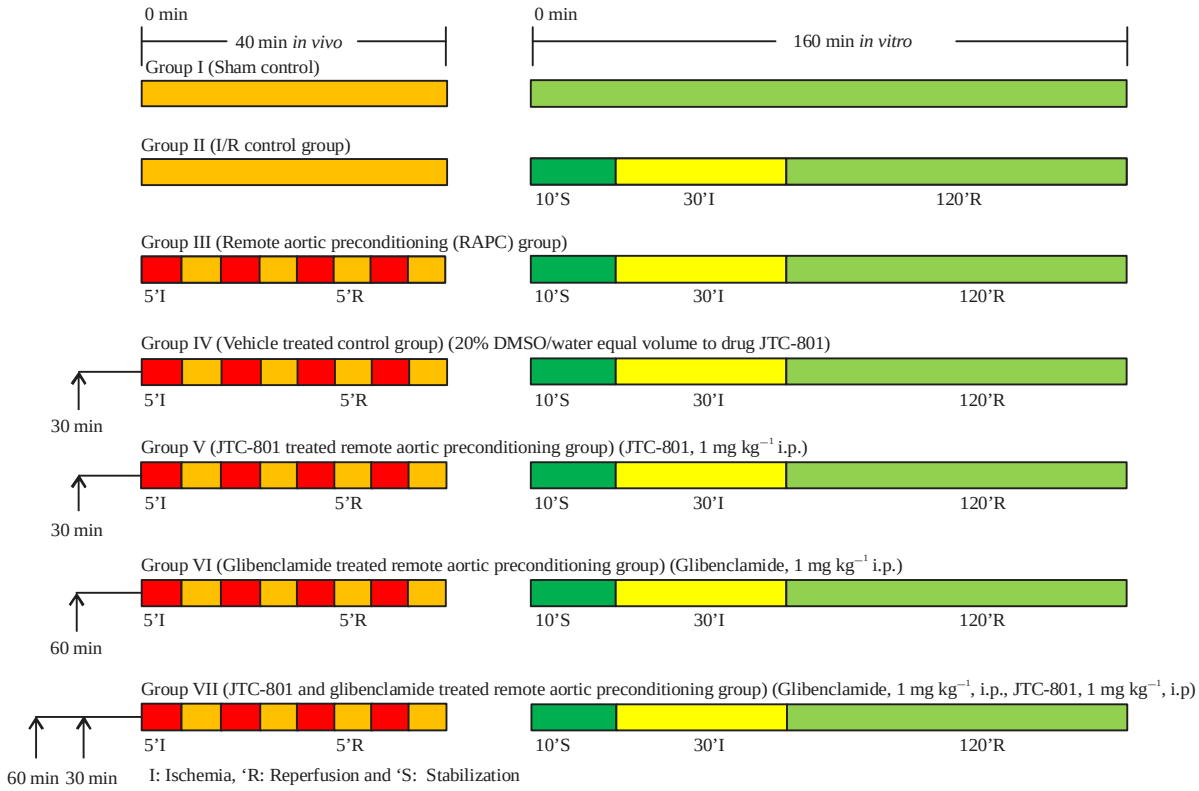

Fig. 1: Diagrammatic representation of experimental protocol

Group 1 (Sham control, n = 6): Rats were subjected to surgical procedure to isolate abdominal aorta and ligature was passed beneath it and aorta was not occluded. Animals were sacrificed after 40 min of isolation of aorta. Hearts were removed and perfused continuously on Langendorff's apparatus for 160 min without subjecting them to global ischemia.

Group 2 (Ischemia/Reperfusion (I/R) control group, n = 6): Rats were subjected to surgical procedure to isolate abdominal aorta and ligature was passed beneath it but aorta was not occluded. Animals were sacrificed after 40 min of isolation of aorta. Hearts were removed and perfused on Langendorff's apparatus and were subjected to 10 min of stabilization followed by 30 min of global ischemia further followed by 120 min of reperfusion.

Group 3 (Remote aortic preconditioning group, n = 6): Rats were subjected to four episodes of remote aortic preconditioning and each episode was comprised of 5 min of ischemia and 5 min of reperfusion. Animals were sacrificed immediately after the last episode of preconditioning. Hearts were removed and perfused on Langendorff's apparatus. Then hearts were subjected to 10 min of stabilization followed by global ischemia for 30 min and reperfusion for 120 min.

Group 4 (Vehicle treated control group, n = 6): About 20% dimethyl sulfoxide (DMSO)/water in equal volume to drug (JTC-801) was administered 30 min before isolation of abdominal aorta. Rest of procedure was same as described in group 3.

Group 5 (JTC-801 treated remote aortic preconditioning group, n = 6): The JTC-801[37,38] (1 mg kg^{-1} i.p.) was administered 30 min before isolation of abdominal aorta. Rest procedure was followed same as described in group 3.

Group 6 (Glibenclamide treated remote aortic preconditioning group, n = 6): Glibenclamide (1 mg kg^{-1} i.p.) was administered 60 min before isolation of abdominal aorta. Rest procedure was followed same as described in group 3.

Group 7 (JTC-801 and glibenclamide treated remote aortic preconditioning group, n = 6): Glibenclamide (1 mg kg^{-1} i.p.) 60 min before and JTC-801 (1 mg kg^{-1} i.p.) 30 min before isolation of abdominal aorta were administered to the rats. Rest procedure was followed same as described in group 3.

Assessment of myocardial injury: Myocardial infarct size was measured macroscopically using TTC staining. Myocardial injury was assessed by the estimation of LDH and CK-MB in the coronary effluent. Both LDH and CK-MB were estimated using commercial kits. Values of LDH and CK-MB were expressed in international units per litre (IU L^{-1}).

Assessment of infarct size: Assessment of myocardial infarct size was done by using triphenyl-tetrazolium chloride (TTC) staining method. Heart was removed from Langenrdorff's apparatus. Both the auricles and the root of aorta were dissected and ventricles were kept overnight at 0°C. Frozen ventricles were sliced into uniform sections of 2-3 mm thickness. The slices were incubated in 1% TTC at 37°C in 0.2 M tris buffer (pH 7.4) for 30 min. The TTC is converted to red formazone pigment by NADH and dehydrogenase enzyme. Therefore, the viable cells stained deep red[39]. The infarcted cells had lost the enzymes and cofactor and thus remained unstained or dull yellow. The ventricular slices were placed between two glass plates. A transparent plastic grid with 100 squares in 1 cm^2 was placed above it. Average area of ventricular slice was calculated by counting the number of squares on either side. Similarly, number of squares falling over non-stained dull yellow area was counted. Infarct size was expressed as percentage of average ventricular volume. Whole of ventricular slices were weighed. Infarcted dull yellow part was dissected out and weighed. Infarct size expressed as a percentage of total ventricular weight.

Estimation of lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) release: Commercially available LDH Enzyme Estimation Kit (Span Dignostic Limited, Surat, Gujrat,India) and CK-MB Enzyme Estimation Kit (Coral Clinical System, Goa, India) were used for the estimation of extent of myocardial injury in coronary flow.

Statistical analysis: All values were expressed as Mean±Standard Deviation (SD). Statistical analysis was performed using Graphpad Prizm Software. The data obtained from the various groups were statistically analyzed using one-way analysis of variance (ANOVA). The *p<0.05 was considered to be statistically significant.

RESULTS

In this study, the myocardial injury was examined in terms of infarct size, LDH release and CK-MB release.

Note: In Fig. 2-7, all 'b' are compared with 'a' and all 'c' are compared with 'b'.

Infarct size: It was observed that sham control group showed a very low infarct size. The I/R control group showed a high degree of infarct size in comparison to sham control group while RAPC treated group showed significant reduction in infarct size (Fig. 2) in comparison to I/R control group.

Vehicle did not attenuate the effect of RAPC. Myocardial infarct size was found to be more in JTC-801 treated animals in comparison to RAPC treated group. Glibenclamide alone attenuated the effect of RAPC in terms of infarct size. Combination of both drugs (JTC-801 and glibenclamide) also attenuated the effect of RAPC and produced high percentage of myocardial infarct size (Fig. 3) in comparison to RAPC treated group of animals.

Estimation of lactate dehydrogenase release: Peak level of LDH release was found at 1 min of reperfusion (Fig. 4). The LDH release was very low in sham control group. The I/R control group showed a high concentration of LDH release at 1 and 30 min of reperfusion. Concentration of LDH was found to be low in case of RAPC treated group in comparison to I/R control group (Fig. 4).

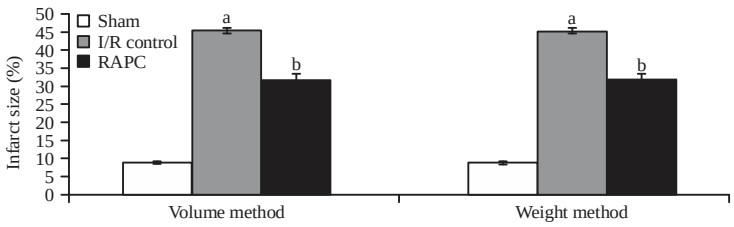

Fig. 2: Effect of remote ischemia preconditioning on myocardial infarct size in isolated rat heart, I/R: Ischemia/Reperfusion control, RAPC: Remote aortic preconditioning, values are expressed as Mean±SD, a: *p<0.05 vs sham control and b: *p<0.05 vs I/R control

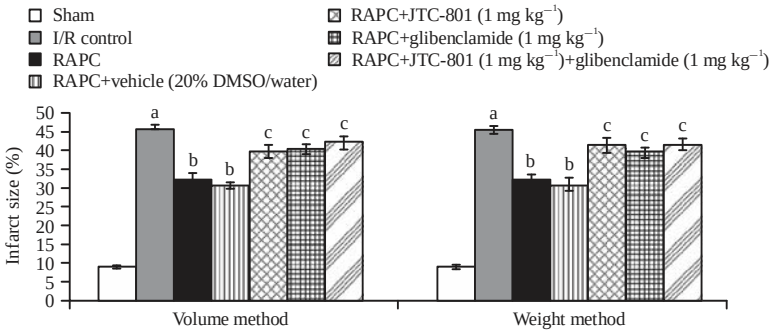

Fig. 3: Effect of pharmacological intervention on myocardial infarct size, I/R: Ischemia/Reperfusion control, RAPC: Remote aortic preconditioning, values are expressed as Mean±SD, a: *p<0.05 vs sham control, b: *p<0.05 vs I/R control and c: *p<0.05 vs RAPC

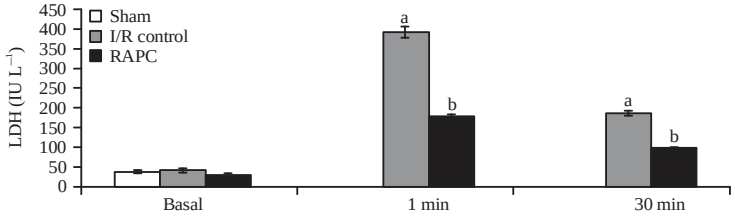

Fig. 4: Effect of remote ischemia preconditioning on release of LDH in isolated rat heart, I/R: Ischemia/Reperfusion control, RAPC: Remote aortic preconditioning, values are expressed as Mean±SD, a: *p<0.05 vs sham control, b: *p<0.05 vs I/R control and c: *p<0.05 vs RAPC

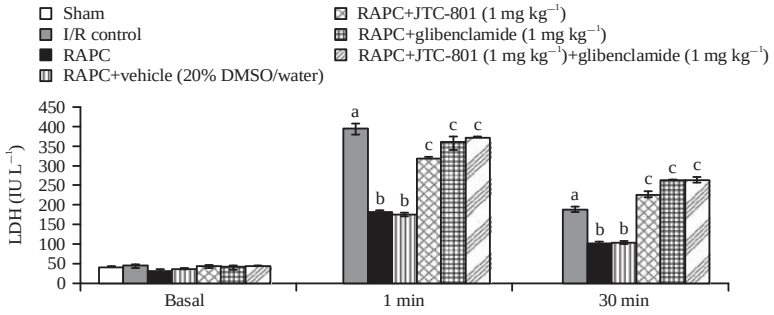

Fig. 5: Effect of pharmacological intervention on release of LDH, I/R: Ischemia/Reperfusion control, RAPC: Remote aortic preconditioning, values are expressed as Mean±SD, a: *p<0.05 vs sham control, b: *p<0.05 vs I/R control and c: *p<0.05 vs RAPC

Vehicle did not raise the concentration of LDH in RAPC treated group, thus did not altered the effect of RAPC. The JTC-801 treated group attenuated the effect of RAPC and produced a high level of LDH release in coronary outflow at 1 and 30 min of reperfusion. Glibenclamide treated group also increased the concentration of LDH release at 1 and 30 min of reperfusion and thus attenutaed the effect of RAPC. Combination of both drugs (JTC-801 and glibenclamide) attenuated the effect of RAPC and increased the concentration of LDH at 1 and 30 min of reperfusion (Fig. 5) in comparison to RAPC treated group.

Estimation of CK-MB release: The CK-MB release was measured at onset and 5 min of reperfusion. The CK-MB release was found to be at its peak level at 5 min of reperfusion (Fig. 6).

The RAPC treated group released a lower concentration of CK-MB in coronary outflow in comparison to I/R control group (Fig. 6).

Vehicle did not affect the release of CK-MB and thus not altered the effect of RAPC. The JTC-801 and glibenclamide when administered alone attenuated the effect of RAPC and increased the concentration of CK-MB in coronary outflow

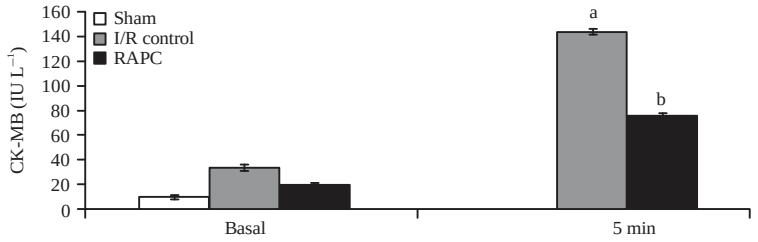

Fig. 6: Effect of remote ischemia preconditioning on release of CK-MB in isolated rat heart, I/R: Ischemia/reperfusion control, RAPC: Remote aortic preconditioning, values are expressed as Mean±SD, a: *p<0.05 vs sham control, b: *p<0.05 vs I/R control and c: *p<0.05 vs RAPC

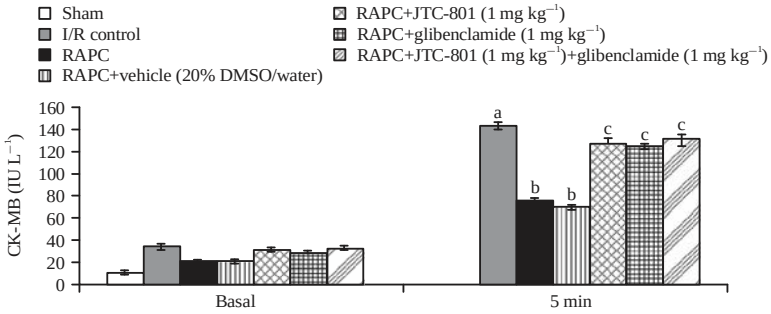

Fig. 7: Effect of pharmacological intervention on release of CK-MB, I/R: Ischemia/Reperfusion control, RAPC: Remote aortic preconditioning, values are expressed as Mean±SD, a: *p<0.05 vs sham control, b: *p<0.05 vs I/R control and c: *p<0.05 vs RAPC

which was significantly high at 5 min of reperfusion as compared to RAPC treated group. Combination of both drugs also attenuated the effect of RAPC and produced a high level of CK-MB in coronary outflow at 5 min of reperfusion (Fig. 7).

Both drugs either given alone in different groups or in combination produced a high degree of myocardial injury in terms of percentage infarct size, LDH release and CK-MB release and attenuated the effect of RAPC.

DISCUSSION

The present study was designed to investigate the possible involvement of ORL_1 receptor in remote aortic preconditioning (RAPC) induced cardioprotection in rat heart. Four episodes each comprising of 5 min of ischemia by occlusion of abdominal aorta and 5 min of reperfusion were employed in the present study to produce remote aortic preconditioning. The release of LDH and CK-MB were estimated in coronary effluent and infarct size was measured to access the effect of RAPC on ischemia and reperfusion induced myocardial injury.

The ORL_1 is an "orphan" receptor which has high degree of structural homology to classical opioid receptors and having very selective endogenous ligand nociceptin[22]. The ORL_1 is widely distributed in brain, spinal cord and in the autonomic nervous system innervated to the heart and blood vessels. During ischemia its expression and activity increases. Evidence indicates nociceptin/orphanin FQ (N/OFQ) may participate in the pathology of cardiac arrhythmias associated with myocardial infarction. Moreover, antagonism of endogenous N/OFQ (selective endogeneous agonist of ORL_1 receptor) produces anti-arrhythmic effects on ventricular arrhythmias in acute myocardial infarction, possibly via modulating PKC activity and action potential of myocytes[40]. It has been reported that ORL_1 activates cAMP/cGMP and subsequently opens K_{ATP} channels[33] which shows cardioprotection[32]. So, this study is designed to see its cardioprotective effect in isolated rat heart using remote aortic preconditioning. In the present study, JTC-801 (ORL_1 receptor antagonist)[37,38] attenuated the cardioprotective effect of remote ischemia preconditioning accessed in terms of myocardial infarct size and release of LDH and CK-MB. Therefore, it may be probable to suggest that cardio protective effect of RAPC may be mediated through ORL_1 receptor.

Expression and activity of ORL_1 gets increase during ischemia insult[34,35]. Nociceptin on binding to ORL_1 receptor shows arteriodilator response by activation of cAMP/cGMP

and subsequent opening of K_{ATP}[34] which shows cardio protection in ischemia injury[32,41]. Glibenclamide is reported to block K_{ATP} channels[12]. In present study, glibenclamide has attenuated remote aortic preconditioning (RAPC) induced cardio protection.

Coadministration of JTC-801 and glibenclamide was unable to produce any additive myocardial injury in RAPC treated group as compared to individual administration of both drugs. Therefore, it may be probable to suggest that cardioprotective effect of RAPC may be mediated through up regulation of ORL_1 receptor and activation of K_{ATP} channels.

CONCLUSION

The JTC-801 and glibenclamide both drugs attenuated the effect of RAPC when administered separately. On the other side, combination of both drugs could not produce and additive effect in comparison to the effect of JTC-801 and glibenclamide alone. On the basis, it may be concluded that cardioprotective effect of remote aortic preconditioning may be mediated through up regulation of ORL_1 receptor and activation of K_{ATP} channels.

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