Possible Role of Opioids and K_{ATP} Channels in Neuroprotective Effect of Postconditioning in Mice

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The present study was designed to investigate the possible role of opioids and K_{ATP} channels in ischemic postconditioning-induced reversal of global cerebral ischemia and reperfusion (I/R) induced neuronal injury. Mice were subjected to global ischemia by bilateral carotid artery occlusion for 10 min followed by reperfusion for 24 h, to produce neuronal injury. Ischemic postconditioning was induced by three episodes of carotid artery occlusion and reperfusion of 10 s each, immediately after global ischemia. Morphine postconditioning was induced by administration of morphine (5 mg/kg i.v.), 5 min prior to reperfusion. Naloxone (5 mg/kg i.v.), opioid receptor antagonist, and glibenclamide (5 mg/kg i.v.), K_{ATP} channel blocker were administered 10 min before global ischemia. Extent of cerebral injury was assessed by measuring cerebral infarct size using triphenyl tetrazolium chloride (TTC) staining. Short-term memory was evaluated using the elevated plus maze test, while degree of motor incoordination was evaluated using inclined beam-walking, rota-rod and lateral push tests. Bilateral carotid artery occlusion followed by reperfusion resulted in significant increase in infarct size, impairment in short-term memory and motor co-ordination. Ischemic/morphine postconditioning significantly attenuated I/R induced neuronal injury and behavioural alterations. Pretreatments with naloxone and glibenclamide attenuated the neuroprotective effects of ischemic/morphine postconditioning. It may be concluded that ischemic/morphine postconditioning protects I/R induced cerebral injury *via* activating opioid receptor and K_{ATP} channel opening.

Key words ischemic postconditioning; cerebral ischemia; opioid; K_{ATP} channel; neuroprotection

Ischemic stroke is a syndrome characterized by rapid onset of neurological injury due to interruption of blood flow to the brain.¹⁾ Extensive research for stroke treatment has been performed in the past several decades; however, few neuroprotectants have been successfully translated from basic research in to clinical application. Thus, stroke remains the leading cause of death due to unavailability of effective strategies for its management.²⁾ Cerebral ischemic preconditioning has been documented to increase ischemic tolerance of cortical neurons and found beneficial in reducing the extent of neuronal injury in stroke.³⁾ However, its clinical applicability has been limited in condition, when the occurrence of stroke is predictable.⁴⁾

Although restoration of blood flow to an ischemic organ is essential to prevent irreversible cellular injury, yet reperfusion *per se* has been noted to augment tissue injury. However, Zhao *et al.*⁵⁾ reported that brief intermittent episodes of ischemia and reperfusion, at the onset of reperfusion after a prolonged period of ischemia, conferred tissue protection and termed the phenomenon as “ischemic postconditioning”. Ischemic postconditioning (IPoC) has been well documented to induce cardioprotection against ischemia/reperfusion (I/R) injury in rats,⁶⁾ mice,⁷⁾ dogs⁵⁾ and rabbits,⁸⁾ *etc.* In addition, a clinical report has also demonstrated postconditioning induced protection to human heart during acute myocardial infarction.⁹⁾ IPoC induced protection against I/R injury has also been reported in brain,^{4,10)} spinal cord,¹¹⁾ kidney¹²⁾ and liver.¹³⁾ Unlike preconditioning, the experimental design of postconditioning allows direct application in the clinical settings to salvage ischemic tissue. Postconditioning may be also used in situations, where preconditioning is difficult or impossible to achieve due to unpredictability of onset of ischemia. Different hypothesis have been proposed by different workers for unfolding the mechanisms of ischemic postconditioning in myocardial protection.^{8,14)} However, the mecha-

nisms involved in IPoC induced protection in brain and other organs have not been well explored.

Morphine is powerful opioid analgesic and widely used in management of acute and chronic pain. Opioid receptor system has also been implicated in the protection of organs during hypoxic and ischemic events.¹⁵⁾ Naloxone has been employed as specific opioid receptor antagonist for investigating the possible involvement of opioids in tissue protection during ischemic events.^{16–18)} Moreover, it has been reported that opioids are possibly involved in ischemic preconditioning and postconditioning induced myocardial protection.^{18,19)} In addition, the involvement of opioid receptors in neuroprotection induced by rapid hypoxic preconditioning has also been documented.²⁰⁾ However, the possible involvement of opioids in IPoC induced neuroprotection is not yet explored.

K_{ATP} channels are found to be present on various organs including heart and brain which play an important role in hypoxia.^{21,22)} There have been reports that suggest the involvement of K_{ATP} channels in ischemic preconditioning,²³⁾ remote preconditioning²⁴⁾ and postconditioning¹⁴⁾ induced protection in heart and remote preconditioning of brain.²⁵⁾ Glibenclamide, a K_{ATP} blocker, has been widely used in management of diabetes and also documented to attenuate cardioprotective effects of morphine preconditioning as well as postconditioning.^{18,26)} However, the role of K_{ATP} channels in ischemic postconditioning induced cerebroprotection is still to be explored. Further, there have been number of reports suggesting the functional inter-relationship between K_{ATP} channels and opioids. K_{ATP} channels have been documented as end effectors for opioids mediated biological actions during ischemia reperfusion, preconditioning and even in post conditioning induced cardioprotection.^{18,27,28)} So, the present study was designed to investigate the possible role of opioids and K_{ATP} channels in ischemic postconditioning induced neuroprotective effects in mice.

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MATERIALS AND METHODS

Animals Male albino swiss mice weighing 25 ± 2 g, maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water were employed for the present study. They were housed in the departmental animal house and were exposed to normal light/dark cycle. The animal experiments were duly approved by Institutional Animal Ethical Committee and were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No.-107/1999/CPCSEA).

Drugs and Chemicals Chloral hydrate (Riedel-deHaen, Germany), morphine (Nalsone pharmaceutical, Amritsar, India) and naloxone (M. S. Pharmachem, Mumbai, India) solutions were prepared in normal saline, while glibenclamide (Zydus Cadila, Ahmedabad, India) was dissolved in DMSO. All other chemicals were obtained from S. D. Fine Chemical, Mumbai, India.

Global Cerebral Ischemia and Reperfusion Mice were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and mid-line ventral incision was made in neck to expose right and left common carotid arteries. After separating from surrounding tissues and vagus nerve, bilateral carotid artery occlusion (BCAO) was performed by cotton thread, passing below carotid arteries, to induce global cerebral ischemia for 10 min. Thereafter, blood was allowed to reflow through carotid arteries.^{29,30} Incision was sutured back in layers. The sutured area was cleaned with 70% ethanol and was sprayed with antibiotic (Neosporin) dusting powder. After completion of surgical procedure, the animals were shifted individually to their home cage and were allowed to recover. All the surgical instruments used in the surgical procedure were sterilized prior to use.

Cerebral Ischemic Postconditioning For the ischemic postconditioning, the carotid arteries were re-occluded for a period of 10 s followed by 10 s of reperfusion. Three such cycles of ischemia and reperfusion were allowed, immediately after 10 min of bilateral carotid artery occlusion.¹⁰

Assessment of Cerebral Infarct Size At the end of 24 h reperfusion after the global cerebral ischemia, animals were sacrificed by cervical dislocation and brains were isolated. The brains were kept overnight at -4°C and frozen brains were sliced into uniform coronal section of about 1 mm thickness. The slices were incubated in 1% triphenyl tetrazolium chloride (TTC) at 37°C in 0.2 M Tris buffer (pH 7.4) for 20 min.³¹ TTC is converted to red formazone pigment by NAD and lactate dehydrogenase and thus stained the viable cells deep red. The infarcted cells have lost the enzyme and cofactor and thus remained unstained dull yellow. The brain slices were placed over glass plate. A transparent plastic grid with 100 squares in 1 cm^2 was placed over it. Average area of each brain slice was calculated by counting the number of squares on either side. Similarly, number of squares falling over non-stained dull yellow area was also counted. Infarcted area was expressed as a percentage of total brain volume. Whole brain slices were weighed. Infarcted dull yellow part was dissected out and weighed. Infarct size was expressed as percentage of total weight of brain. Percentage infarct size was calculated by both volume and weight method as de-

scribed earlier.¹⁷⁾

Short Term Memory Evaluation Using Elevated Plus Maze Plus maze consisted of two open (16×5 cm) and two enclosed ($16 \times 5 \times 12$ cm) arms, connected by a central platform (5×5 cm). The apparatus was elevated to a height of 25 cm above the floor. A fine line was drawn in the middle of the floor of each enclosed arm. All the animals were given a single trial/day on plus maze. Each mouse was individually placed at the end of open arm facing away from central platform of the maze. The time taken by the mouse to enter from open arm into the enclosed arm, with all the four legs was taken as transfer latency time (TLT). In case, the animal did not enter the enclosed arm within 90 s, it was gently pushed into the enclosed arm and TLT of 90 s was assigned to it. The animal was allowed to explore the maze for an additional 10 s after the measurement of TLT.^{32,33)}

The animals were subjected to three such trials on elevated plus maze for three consecutive days.³⁴⁾ TLT recorded on the 3rd day served as an index of acquisition. After three days training, animals were subjected to global cerebral ischemia for 10 min followed by reperfusion for 24 h. Thereafter, TLT was recorded on 4th day (24 h after ischemia) which served as an index of memory. Utmost care was taken not to change the relative location of plus maze with respect to any object serving as visual clue in laboratory.

Inclined Beam-Walking Test Inclined beam-walking test was employed to evaluate fore and hind limb motor coordination.^{10,35)} Each animal was individually placed on a metallic bar 55 cm long and 1.5 cm wide, inclined at an angle of 60° from ground. Scale ranging from 0 to 4 was used to grade the motor performance. A grade of 0 was assigned to animal that could readily traverse the beam, grade 1 was given to animal demonstrating mild impairment, grade 2 was assigned for moderate impairment, grade 3 was given for severe impairment and grade 4 was assigned to animal completely unable to walk on the beam. Inclined beam-walking test was performed before global cerebral ischemia and 24 h after global cerebral ischemia/reperfusion.

Rota-Rod Test Rota-rod has been used to evaluate motor coordination by testing the ability of mice to remain on revolving rod.³⁶⁾ The apparatus consisted of horizontal rough metal rod of 3 cm diameter attached to a motor with variable speed. This 70 cm long rod was divided into four sections by wooden partitions. The rod was placed at a height of 50 cm to discourage the animals to jump from the rotating rod. The rate of rotation was adjusted to allow the normal mice to stay on it for five minutes. Each mouse was given five trials before the actual reading was taken. The animals staying on revolving rod for period of five minutes before the surgical procedure were selected and the test was again performed 24 h after global cerebral ischemia and reperfusion.

Lateral Push Test Motor coordination was also evaluated by observing the percentage of mice showing resistance to lateral push.^{30,37)} A mouse was placed on a rough surface for firm grip and evaluated for resistance to lateral push from either side of shoulder. The test was performed before global cerebral ischemia and 24 h after global cerebral I/R. Mice with increased or decreased resistance to lateral push after global ischemia were assigned + or - score, respectively.

Experimental Protocol Ten groups, each comprising of six male swiss albino mice, were employed in the present

study.

Group I (Sham Group): Mouse was subjected to surgical procedure to expose carotid arteries and thread was passed below both the arteries, without any occlusion. After 10 min, animal was sutured back after removal of thread and allowed to recover for 24 h.

Group II (Control Group): Mouse was subjected to surgical procedure to expose right and left carotid arteries. Thread was passed below both the arteries and global ischemia was produced for 10 min by occluding both arteries. It was followed by reperfusion for 24 h.

Group III (Ischemic Postconditioning Group): Mouse was subjected to 10 min global cerebral ischemia followed by three episodes of ischemia and reperfusion of 10 s each, after which 24 h reperfusion period was permitted.

Group IV (Morphine Postconditioning Group): Mouse was subjected to 10 min global ischemia and 24 h reperfusion as described in group II. Morphine (5 mg/kg i.v.) was administered 5 min prior to reperfusion for pharmacological postconditioning.

Group V (Naloxone in Control Group): Naloxone (5 mg/kg i.v.) was administered 10 min prior to global cerebral ischemia and rest of protocol was same as described in group II.

Group VI (Naloxone in Ischemic Postconditioning Group): Naloxone (5 mg/kg i.v.) was administered 10 min prior to global cerebral ischemia and rest of protocol was same as described in group III.

Group VII (DMSO in Control Group): DMSO (10 ml/kg i.v.) was administered 10 min prior to global cerebral ischemia and rest of protocol was same as described in group II.

Group VIII (Glibenclamide in Control Group): Glibenclamide (5 mg/kg i.v.) was administered 10 min prior to global cerebral ischemia and rest of protocol was same as described in group II.

Group IX (Glibenclamide in Ischemic Postconditioning Group): Glibenclamide (5 mg/kg i.v.) was administered 10 min prior to global cerebral ischemia and rest of protocol was same as described in group III.

Group X (Glibenclamide in Morphine Postconditioning Group): Glibenclamide (5 mg/kg i.v.) was administered 10 min prior to global cerebral ischemia and rest of protocol was same as described in group IV.

Statistical Analysis Statistical analysis for infarct size and TLT was done using one-way ANOVA followed by Tukey's multiple range test for *post-hoc* analysis. Statistical significance for inclined beam-walking test and rota-rod test were calculated using Wilcoxon rank sum test, while for lateral push test, chi square test was employed. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Effect of Pharmacological Interventions on Cerebral Infarct Size Global cerebral ischemia of 10 min followed by reperfusion for 24 h (I/R) produced significant increase in cerebral infarct size measured by both volume and weight method. Ischemic postconditioning significantly attenuated I/R induced increase in cerebral infarct size. Pharmacological postconditioning with morphine (5 mg/kg i.v.), 5 min

prior to reperfusion also attenuated I/R induced cerebral infarct size.

Naloxone (5 mg/kg, i.v.) *per se* did not affect I/R induced cerebral infarct size. However, it significantly abolished ischemic postconditioning induced decrease in cerebral infarct size. Moreover, Glibenclamide (5 mg/kg, i.v.) *per se* did not affect I/R induced cerebral infarct size. However, it significantly abolished ischemic postconditioning and morphine postconditioning induced decrease in cerebral infarct size (Figs. 1, 2).

Effect of Pharmacological Interventions on Impairment of Short-Term Memory Sham group animals showed a significant decrease in day 4 Transfer Latency Time (TLT) as compared to their day 3 TLT. Global cerebral ischemia followed by reperfusion significantly prevented the decrease in day 4 TLT, reflecting impairment of memory. Ischemic postconditioning significantly attenuated I/R-induced increase in day 4 TLT indicating reversal of I/R-induced impairment of memory. Pharmacological postconditioning with morphine (5 mg/kg i.v.), 5 min prior to reperfusion also attenuated I/R induced impairment of memory.

Naloxone (5 mg/kg, i.v.) *per se* did not modulate I/R-in-

Fig. 1. Photographs of Brain Sections Stained with TTC of Different Groups A (Sham), B (Control), C (Ischemic Postconditioning), D (Morphine Postconditioning), E (Naloxone in Ischemic Postconditioning), F (Glibenclamide in Ischemic Postconditioning), G (Glibenclamide in Morphine Postconditioning)

Fig. 2. Effect of Pharmacological Interventions on Global Cerebral Ischemia and Reperfusion-Induced Cerebral Infarct Size by Volume and Weight Method

Values are mean $\pm$ S.E.M. for six animals. ^a $p < 0.05$ vs. Sham, ^b $p < 0.05$ vs. Control, ^c $p < 0.05$ vs. IPoC/MPoC ($F = 145.6$ for volume and 185.4 for weight method). IPoC: Ischemic Postconditioning, MPoC: Morphine Postconditioning, Nal: Naloxone, Gli: Glibenclamide, DMSO: Dimethyl Sulfoxide.

Fig. 3. Effect of Pharmacological Interventions on Global Cerebral Ischemia and Reperfusion-Induced Impairment of Short-Term Memory

Values are mean $\pm$ S.E.M. for six animals. ^a $p < 0.05$ 4th day TLT of Sham vs. 3rd day TLT of Sham, ^b $p < 0.05$ vs. 4th day TLT of Sham, ^c $p < 0.05$ vs. 4th day TLT of Control, ^d $p < 0.05$ vs. 4th day TLT of IPoC/MPoC ($F = 2.433$ for 3rd day and 263.1 for 4th day).

duced increase in day 4 TLT. However, it significantly attenuated IPoC induced decrease in day 4 TLT. Moreover, Glibenclamide (5 mg/kg, i.v.) also abolished the ischemic postconditioning and morphine postconditioning mediated attenuation of I/R-induced increase in day 4 TLT of control (Fig. 3).

Effect of Pharmacological Interventions on Motor Incoordination Score by Inclined Beam Walking Test Global cerebral ischemia of 10 min followed by reperfusion for 24 h produced significant rise in motor incoordination score measured by inclined beam walking test, after 24 h of reperfusion as compared to sham group. Ischemic postconditioning significantly reduced I/R induced increase in motor incoordination score. Pharmacological postconditioning with morphine (5 mg/kg i.v.), 5 min prior to reperfusion, also attenuated I/R induced increase in motor incoordination score (Fig. 4).

Naloxone (5 mg/kg) *per se* did not affect I/R-induced motor incoordination. However, it significantly attenuated IPoC-induced decrease in motor incoordination. Moreover, Glibenclamide (5 mg/kg, i.v.) *per se* did not affect I/R-induced motor incoordination. However, it significantly attenuated IPoC and morphine postconditioning-induced decrease in motor incoordination (Fig. 4).

Effect of Pharmacological Interventions on Motor Incoordination by Rota-Rod Test Global cerebral ischemia of 10 min followed by reperfusion for 24 h produced significant reduction in fall down time, measured by rota-rod test, after 24 h of reperfusion as compared to sham group. Ischemic postconditioning significantly increased I/R induced reduction in fall down time. Pharmacological postconditioning with morphine (5 mg/kg i.v.), 5 min prior to reperfusion, also attenuated I/R induced reduction in fall down time.

Naloxone (5 mg/kg i.v.) *per se* did not modulate I/R-induced fall down time. However, it significantly attenuated IPoC-induced increase in fall down time. Moreover, Glibenclamide (5 mg/kg i.v.) *per se* did not modulate I/R-induced fall down time. However, it significantly attenuated IPoC and morphine postconditioning-induced increase in fall down time (Fig. 5).

Effect of Pharmacological Interventions on Resistance

Fig. 4. Effect of Pharmacological Interventions on Global Cerebral Ischemia and Reperfusion-Induced Impairment of Motor Performance Measured by Inclined Beam-Walking Noted after 24 h of I/R

Values are mean $\pm$ S.E.M. for six animals. ^a $p < 0.05$ vs. Sham, ^b $p < 0.05$ vs. Control, ^c $p < 0.05$ vs. IPoC/MPoC ($F = 140.2$).

Fig. 5. Effect of Pharmacological Interventions on Global Cerebral Ischemia and Reperfusion-Induced Impairment of Motor Performance Measured by Rota-rod Test Noted after 24 h of I/R

Values are mean $\pm$ S.E.M. for six animals. ^a $p < 0.05$ vs. Sham, ^b $p < 0.05$ vs. Control, ^c $p < 0.05$ vs. IPoC/MPoC ($F = 154.1$).

to Lateral Push Response Global cerebral ischemia of 10 min followed by reperfusion for 24 h produced a significant decrease in percentage of mice exhibiting resistance to lateral push in mice noted after 24 h of reperfusion, when compared to sham group. Ischemic postconditioning significantly attenuated I/R induced decrease in percentage of mice demonstrating resistance to lateral push as compared to the control group. Pharmacological postconditioning with morphine (5 mg/kg i.v.) administered 5 min prior to reperfusion also attenuated I/R induced decrease in percentage of mice demonstrating resistance to lateral push as compared to the control group.

Naloxone (5 mg/kg i.v.) *per se* did not affect I/R-induced decrease in percentage of mice demonstrating resistance to lateral push. However, it significantly attenuated IPoC-induced increase in fall down time. Moreover, Glibenclamide (5 mg/kg i.v.) *per se* did not affect I/R-induced decrease in percentage of mice demonstrating resistance to lateral push. However, it significantly attenuated IPoC and morphine postconditioning-induced increase in percentage of mice demonstrating resistance to lateral push response (Fig. 6).

DISCUSSION

Global cerebral ischemia/reperfusion (I/R) model, employed in the present study, has been reported to simulate the clinical situation of cerebral ischemia due to cardiac arrest and drowning.³⁸ Hippocampus is involved in regulation of

Fig. 6. Effect of Pharmacological Interventions on Global Cerebral Ischemia and Reperfusion-Induced Impairment in Resistance to Lateral Push Noted after 24 h of I/R

^a $p < 0.05$ vs. Sham, ^b $p < 0.05$ vs. Control, ^c $p < 0.05$ vs. IPoC/MPoC ($F = 192.8$).

memory, short term as well as long term and global cerebral I/R is reported to produce short term memory deficits through deleterious effects on hippocampus.³⁹⁾ Therefore, elevated plus-maze test was employed to assess short-term memory deficits associated with I/R injury. Further, cerebral ischemia is documented to impair sensorimotor ability.⁴⁰⁾ So, inclined beam walking, rota-rod and resistance to lateral push tests were employed for evaluating sensorimotor disturbances due to I/R injury. Extent of I/R induced neuronal injury, in term of cerebral infarction, was assessed using triphenyl tetrazolium chloride (TTC) staining.³⁰⁾

In the present investigation, global I/R produced significant neuronal injury, short term memory impairment and sensorimotor disturbances, which are in line with earlier reports of our laboratory.^{17,25,30,33)} However, ischemic postconditioning (IPoC) attenuated I/R induced memory deficits indicating its protective effects on hippocampal neurons. Earlier, it has been reported that IPoC reduces I/R induced hippocampal damage.^{41,42)} Further, IPoC also attenuated I/R induced impairment in sensorimotor functions, which may possibly due to its protective effects on cortical neurons and striatum. Earlier, IPoC has been documented to prevent I/R induced damage to cortical neurons and striatum.⁴²⁾ Collectively, these protective effects of IPoC on hippocampus, cortex and striatum were manifested in the form of reduction in cerebral infarct size, measured by both volume and weight methods. Moreover, there was a direct correlation between the degree of infarction and impairment in behavioral functions (memory and sensorimotor), as already reported in earlier papers from our laboratory.^{17,30)} The noted neuroprotective effects of IPoC are consistent with earlier report from our laboratory¹⁰⁾ and from others.^{4,42)} The concept of interrupted, reperfusion, *i.e.*, ischemic postconditioning, initially given by Zhao *et al.*⁵⁾ for cardioprotection has been extended and reported to markedly reduce cell death in brain,^{4,10)} liver¹³⁾ and kidney¹²⁾ during I/R injury. However, unlike in cardioprotection the mechanisms of IPoC induced neuroprotection are not unfolded.

In the present study, administration of naloxone *per se* did not modulate I/R induced neuronal injury, which is in line with earlier report from our laboratory,¹⁷⁾ however it abolished IPoC induced protective effects suggesting the possible involvement of endogenous opioids in IPoC induced neuroprotection. Further, pharmacological postconditioning with morphine also produced neuroprotection similar to that of

IPoC. It further supports the contention that release of endogenous opioids during IPoC may be responsible for neuroprotection. The opioid receptor system has also been implicated in the protection of organs during hypoxic and ischemic events.¹⁵⁾ Recently, it has been reported by our laboratory that opioids release is playing an important role in remote mesenteric preconditioning induced cerebroprotective effects.¹⁷⁾ The involvement of opioids has also been reported in IPoC induced protection in heart.¹⁶⁾ However, perhaps it is first report demonstrating such involvement in IPoC induced neuroprotection.

Administration of glibenclamide *per se* did not modulate I/R induced neuronal injury. However, it abolished neuroprotective effect of IPoC and morphine postconditioning suggesting that K_{ATP} channel opening may be the end effector of opioids mediated neuroprotection in IPoC. Opening of K_{ATP} channels has been reported as an end-effector of number of mediators triggered in preconditioning as well as in postconditioning induced cardioprotection.^{18,26,27)} Opioids are also reported to mediate their cardioprotective effects in IPoC *via* opening of K_{ATP} channels.¹⁸⁾ It has been described that opioid receptor activation leads to burst of O_2 derived free radicals and result in opening of K_{ATP} channels.²⁷⁾ Also, the evidences have shown that μ and δ opioid receptors are linked to K^+ channels.²⁸⁾ So based on the results of present study, it may be suggested that opioid receptor mediated K_{ATP} channel opening is responsible for neuroprotection in ischemic/morphine postconditioning.

Further, opioid receptors activation has been known to stimulate phosphoinositide 3-kinase (PI3K) pathway.^{43,44)} It has also been demonstrated that the protective effect due to activation of PI3K pathway are mediated through K_{ATP} channel opening.⁴³⁾ Moreover, a key role of PI3K pathway in IPoC induced neuroprotection has been documented by our laboratory.¹⁰⁾ So, it may be proposed that release of opioids during postconditioning with subsequent activation of PI3K pathway and opening of K_{ATP} channel is responsible for neuroprotective effects. However, further studies are needed to elucidate the relationship between opioid receptors activation, PI3K pathway stimulation and opening of K_{ATP} channels in neuroprotective effects of postconditioning. Moreover, further study shall be warrant to identify the opioids receptor subtypes involved in IPoC induced cerebroprotection. Nevertheless, it is the first report demonstrating the involvement of opioids and K_{ATP} channels in ischemic postconditioning induced cerebroprotection against I/R injury in mice.

CONCLUSION

It may be concluded that cerebral ischemic postconditioning induced neuroprotective effects against I/R induced neuronal injury are mediated through the release of endogenous opioids with subsequent opening of K_{ATP} channels.

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