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Possible involvement of Opioid like Receptor1 (ORL1) Receptor in Ischemic Preconditioning Induced Protection in Rat Brain

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ABSTRACT

Opioid receptor like 1 receptor (ORL1) is a family of G-protein coupled receptor, reported to produce cardio-protection against ischemia-reperfusion induced injury in rat heart. The present study has been designed to investigate the role of ORL1 receptor in ischemic preconditioning (IPC) induced protection in rat brain. IPC was induced by giving 3 episodes of ischemia and reperfusion. Global ischemia for 17 min was given by occlusion of carotid artery followed by 24hr of reperfusion. Memory was assessed by measuring escape latency time (ELT) for consecutively 4 days and time spent in target quadrant (TSTQ) was measured on 5th day by using morrish water maze. IPC significantly decrease in infarct size and improvement in memory as compared to ischemia/reperfusion (I/R) in control group. Pretreatment with JTC-801(1mg/kg, *i.p.*), a selective ORL1 receptor antagonist or Glibenclamide (1mg/kg, *i.p.*), the KATP channel blocker significantly reduce the amplitude of IPC induced cerebroprotection measured in terms of infarct size and positively affects the memory measured in terms of time spent in target quadrant (TSTQ) at 5th day as compare to IPC group. The cerebroprotective effect of IPC was significantly attenuated in JTC-801 and Glibenclamide in combination as compare to Ischemic preconditioning (IPC) group and treated group (Glibenclamide). These results may indicate that cerebroprotective effect of IPC of brain and improvement of memory mediated through ORL1 receptor and activation of KATP channels.

Keywords: Opioid receptor like1 receptor, ischemic preconditioning, JTC-801, latency time

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INTRODUCTION

Stroke is a cerebrovascular accident (CVA) characterized by rapidly lossed brain function due to disturbance in blood supply (Fagan et al., 2005) which is due to ischemia caused by thrombosis, embolism or hemorrhage (Sims et al., 2009) thus affected areas of brain are unable to function properly and ultimately died (Fagan et al., 2005) . Ischemic preconditioning (IPC) is a strong endogenous protective phenomenon of short alternative cycles of sub lethal ischemia followed by reperfusion before the subsequent prolonged ischemic insult which improves the tolerance against injury due to ischemia reperfusion (Fagan et al., 2005; Murry et al., 1986; Tomai et al., 1999). Cerebral ischemic preconditioning is an endogenous neuroprotective phenomenon in which a noxious stimulus applied to brain confers transient protection against a subsequent ischemic insult which is injurious every time (Gidday et al., 2006). Orphan receptor having high degree of homology to the "classical" opioid receptors on structural origin has been termed as Opioid receptor like 1 (ORL1) (Mollereau et al., 1994). The physiological function on which all emphasis has been taken is totally dependent on anatomical distribution of the ORL1 receptor (Mollereau et al., 2000).

ORL1 receptor is widely distributed in the CNS particularly in the forebrain, in both the dorsal and ventral horns of spinal cord and throughout the brain stem (Salis et al., 2000). ORL1 receptor produce cardioprotection against ischemia reperfusion induced injury in rat heart (Suzuki et al., 2003; Lavania et al., 2013). It has been documented that ORL1 receptor get upregulated during ischemic insult (Malinowska et al., 2002; Guo et al., 2008) and recently it has been reported in the laboratory that ischemic precondition produced protection of heart against ischemia-reperfusion induced injury by ORL1 receptor (Guo et al., 2008). This study was designed to elaborate and investigate the role of

opioid receptor like 1 (ORL1) receptor plays in ischemic preconditioning induced protection in the brain of rat.

MATERIALS AND METHODS

Experimental Animals

In the behavioral study for Ischemic preconditioning of brain, age matched albino wistar rats, weighing about 220-250g have been employed in present research. Animals were kept in polypropylene cages with husk bedding and were feed on standard chow diet with water ad libitium and a semi-sound proof laboratory was selected to carry out all behavioral assessments in between 9.00 AM to 06.00 PM. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) in accordance with the national guidelines on the use of laboratory animals (GLAIPR/CPCSEA/IAEC/2014/P.C01/R01). All the animals selected for this experiment were taken from age-matched group of rats to avoid the variability among these experimental groups and procured from animal house of Institute of Pharmaceutical research, GLA University, Matura, Uttar Pradesh, India (1260/P0/ac/09/CPCSEA).

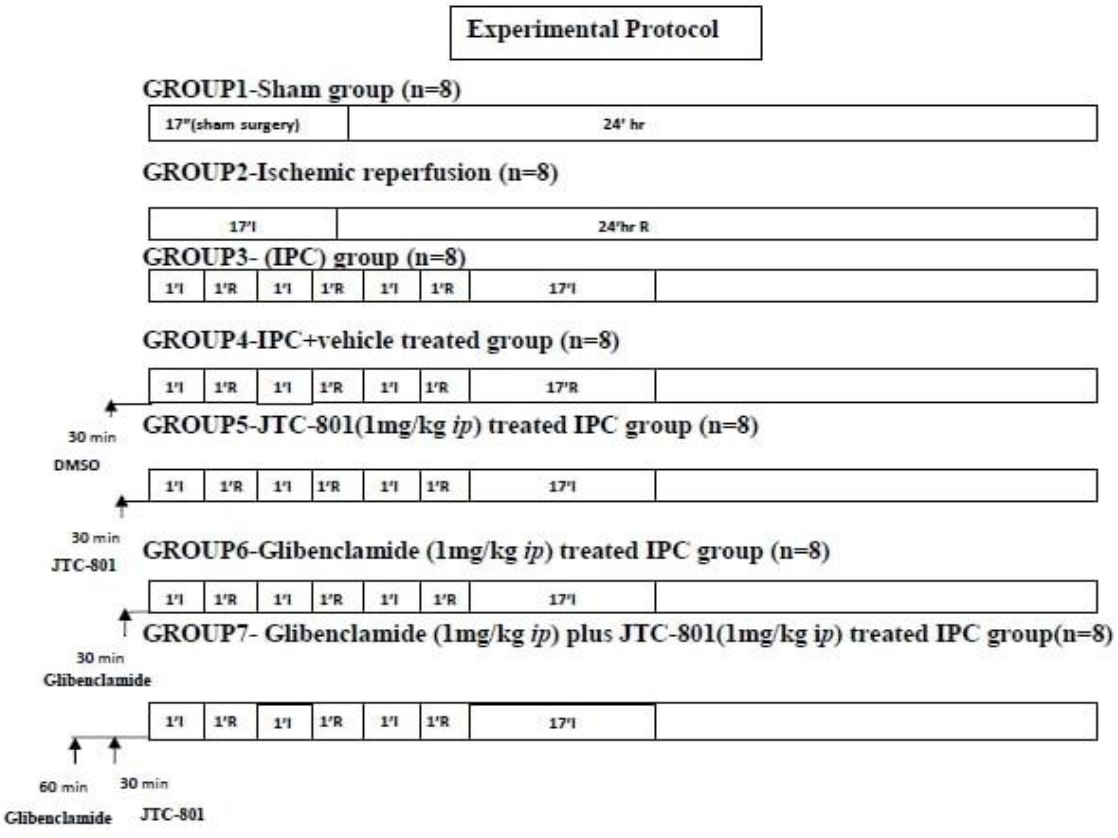
Drugs and Chemicals

JTC-801 (Sigma-Aldrich, Bangalore, India) and Glibenclamide (Medrose drug and pharmaceuticals Pvt. Ltd, India) were dissolved individually in DMSO solution before use. 1% solution of TTC stain (CDH Pvt. Ltd, New Delhi) was prepared in Tris-chloride buffer (CDH Pvt. Ltd., New Delhi), pH was adjusted to 7.4 pH and was used to measure cerebral infarct size. All other reagents used in present investigation were taken of analytical grade and freshly prepared (CDH Chemicals, Delhi).

Ischemic Brain Preconditioning

Rats were anesthetized using thiopental sodium (30 mg/kg, *i.p.*) (Kushwaha et al., 2011). Left and right common carotid arteries were exposed by a midline ventral incision in the neck which will isolated from the vagus nerve and surrounding tissue. A shoelace knot was tied with cotton thread by passing it below the each of carotid arteries and after 17 min of global cerebral ischemia, reperfusion was allowed for 24 h and incisions were sutured in layers (Himori et al., 1990; Rehni et al., 2008). The sutured area was cleaned with 70% ethyl alcohol and then sprayed with antiseptic dusting powder. The animals were shifted separately to their home cage and were allowed to recover overnight; finally behavioral assessment of the animals was done instantaneously before surgical procedure as well as 24 h after the surgery. The animals were kept on heating pad during surgery in order to uphold the body temperature to avoid the effect of temperature variations on the results obtained after completion of study. The carotid arteries were occluded for 1 min followed by reperfusion for 1 min, for previous episodes of ischemic preconditioning and three such cycles of ischemia and reperfusion were perform before bilateral carotid artery occlusion (Rehni et al., 2008., 2011). A single dose of JTC-801, a ORL1 antagonist drug (1mg/kg; *i.p.)* (Rawls et al., 2007) was given 30 min and/or Glibenclamide (1mg/kg *i.p.)* (Dubey et al., 2012) was given 60 min before the ischemic preconditioning. For assessment of memory, Morris water maze (MWM) test was performed (Rehni et al., 2008).

Experimental Protocol



(I means ischemia, R means reperfusion)

GROUP I–(Sham group n=8): Rats were subjected to surgical procedure isolate the carotid arteries and a cotton thread was passed below it in such a way that arteries were not occluded in any extant. After 17 min, threads were removed and animal was sutured back then allowed to recover for a time period of 24 h.

GROUP II-(Ischemia Reperfusion (I/R) group; n=8): Each rat was subjected to 17 min of global cerebral ischemia followed by reperfusion for 24 h.

GROUP III-(Ischemic preconditioning group; n=8): Three episodes comprising 1 min occlusion and 1 min reperfusion shall be followed by 17 min global ischemia then 24hr of reperfusion.

GROUP IV-(Vehicle Treated Group; n=8): Rats were administered vehicle i.e. dimethyl sulfoxide (DMSO) 30 minutes before ischemic preconditioning and rest of procedure was same as described in group III.

GROUP V-(JTC-801 (1mg/kg; i.p.): Treated Ischemic preconditioning group; **n=8**): JTC-801 (1mg/ kg*; i.p*) (Rawls et al., 2007) was administered 30 min before preconditioning and rest of procedure was same as described in group III.

GROUP VI-(Glibenclamide 1mg/kg; i.p.): Treated Ischemic preconditioning group; n=8): Glibenclamide (1mg/kg;*i.p.*) (Dubey et al., 2012) was administered 60 minutes before the preconditioning and rest procedure was same as described in group III.

GROUP VII–(JTC-801 and Glinenclamide treated Ischemic preconditioning group; n=8):

Rats were administered Glibenclamide (1mg/kg*; i.p.*) (Dubey et al., 2012) 60 minutes before and JTC-801(1 mg/kg *i.p.*) (Rawls et al., 2007)30 minutes before the preconditioning and rest procedure was followed same as described in group III.

Assessment of infract size

At the end or 24 hr of reperfusion after global cerebral ischemia, animals were sacrificed by spinal dislocation brain was removed and placed immediately in ice cold saline for 10 min. Brain samples were sliced into identical coronal section of about 1mm thickness and incubated in 1% of 0.2 M Tris buffer of pH 7.4 at 37^{0}C for 20 min (Rehni et al., 2008; Bochelen et al., 1999) then red formazone pigment obtained from conversion of triphenyltetrazolium chloride (TTC) by NAD and dehydrogenase enzyme was used to stain the viable cells to deep red. The infracted cells remain unstained dull yellow because they lose the enzyme and co-factor. A visible plastic grid with 100 squares of 1cm^2 area was placed over the brain slices which were kept in a glass plate. The number of squares on either side of plastic grid was counted for calculating the average area of each brain slice. Similarly the yellow area was also calculated by transparent plastic grid method and whole brain slices were weighed and Infracted area was expressed as a percentage of total brain volume. Percentage of total wet weight of brain was expresses as the infracted dull yellow portion (Rehni et al., 2011).

Statistical Analysis

All values were expressed as mean ± standard deviation (S.D). Standard analysis was done by using graph pad prism software. The data obtained from the various groups were statistically analyzed by using one-way analysis of variance (ANOVA) and *P < 0.05 was considered as statistically significant.

Assessment of memory and learning using the Morris water maze (MWM)

The Morris Water Maze, a circular pool having water used to train the rats or mice by using extra-maze visual clues to locate an escape platform hidden beneath the surface of opaque water (Morris., 1984).The visible platform version of Morris Water Maze method is used to estimates the non-hippocampal tasks, disrupted by dorsal striatum lesions of brain but hidden platform method is used for spatial memory which is very sensitive to hippocampal damage (McDonald et al., 1994).

It also concerned that water maze may be used to induce anxiety or fear in mice, suggesting involvement of amygdale as well as hippocampus (Akirav et al., 2001). Learning and memory in animals is assessed by Morrish water maze test which is a swimming based model where animals learn to escape on a hidden platform. Morrish water maze contains a large circular tank of 45 cm height with 150 cm in diameter and filled with opaque water to a depth of 30 cm, maintained at temperature of 28±1°C. Water was made opaque with white colored non-toxic dye. Tank was divided into four chambers which were equal in size with the help of two threads fixed at right angles to each other on a rim of tank. A white platform of 10 cm^2 area was submerged in tank and placed inside the target quadrants below 1 cm surface of water. The position of platform must not be changed during whole training sessions and estimation experiments.

Each animal was subjected to four successive training trials on each day with inter trial gaps of 5 min. The animal was gently placed in opaque water between quadrants, facing the wall of tank at every trial drop location keep changing then animal was allowed 120 sec of time to locate underwater platform. When the platform was found by animal, it was allowed to stay on the platform for 20 sec and if failed to find the platform within 120 sec, it was guided gently onto the platform and allowed to stay there for 20 sec. The escape latency time (ELT) to locate the hidden platform in water maze was noted on 4th day as index of learning and memory. Each animal was subjected to four training trials on each day for four consecutive days. Starting position of animal was changed with each exposure as mentioned below. The target quadrants (Q4) remain constant during entire training periods like Day1-Q1 Q2 Q3 Q4, Day2-Q2 Q3 Q4 Q1, Day3-Q3 Q4 Q1 Q2 and Day4-Q4 Q1 Q2 Q3.

On fifth day of study the platform was removed. The study was repeated for each mouse by allowing exploring the tank for 120 sec and recording the mean time spent in all quadrants. The mean time spent by animal in the target quadrant searching hidden platform was noted as an index of memory. The experimenter will always stand at the same position. The special care was taken about visual clues in the laboratory which can helps to find the relative location in water maze during the total duration of study. All of trials were completed between 09:00 AM to 06:00 PM (Morris., 1984; Sharma et al., 2008).

RESULTS

Effect of treatment/procedure on ischemia and reperfusion induced cerebral infarct size

1. **Effect of ischemia and reperfusion on infarct size of rat brain.**

Global ischemia of 17 minutes followed by reperfusion of 24 hr significantly increased the brain infarct size as compared to sham control group (Slide 2, Figure 1, and Table 1).

2. Effect of ischemic preconditioning on injury in rat brain.

Three episodes of IPC each episode consist of 1 min of ischemia and 1 min of reperfusion significantly reduced I/R induced increase in infarct size of rat brain (Slide 3, Figure 1, Table 1).

3. Effect of IPC induced protective effect on brain in vehicle pretreated rat.

Administration of vehicle 30 min prior to the IPC each episode consist of 1 min of ischemia and 1 min of reperfusion could not affect protective effect of IPC in terms of infarct size (Slide4, Figure 1, Table 1).

4. Effect of IPC induced protective effect on brain in JTC-801(1mg/kg, *i.p.)* pretreated rat.

Administration of JTC-801 (1mg/kg, *i.p.)* 30 minutes before the three episodes of IPC each episode consist of 1min of ischemia and 1 min of reperfusion significantly abolished the restoration of IPC induced protection noted in terms of increase in infarct size (Slide 5, Figure 1, Table 1).

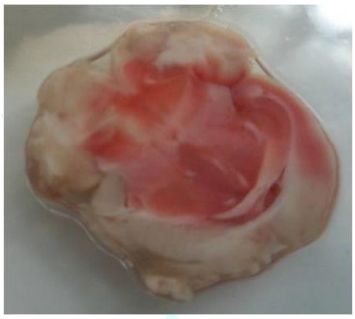
5. Effect of IPC induced protective effect on brain in Glibenclamide (1mg/kg, *i.p.*)

Administration of Glibenclamide (1mg/kg *i.p.)* 60 minutes before the three episodes of IPC each episode consist of 1 min of ischemia and 1 min of reperfusion significantly abolished the restoration of IPC induced protection noted in terms of increase in infarct size (Slide 6, Figure 1, Table 1).

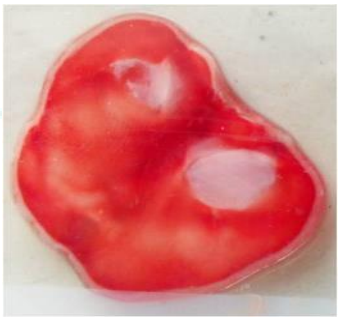
6. Effect of IPC induced protection in JTC-801(1mg/kg; *i.p.)*and Glibenclamide (1mg/kg, *i.p.*) pretreated rat brain.

Administration of JTC-801 (1mg/ kg, *i.p.)* 30 minutes before and Glibenclamide (1mg/kg *i. p.*) 60 minutes before the three episodes of IPC each episode consist of 1 min of ischemia and 1 min of reperfusion were produce additional effect on neuronal injury noted in terms of infarct size (Slide 7, Figure 1, Table 1).

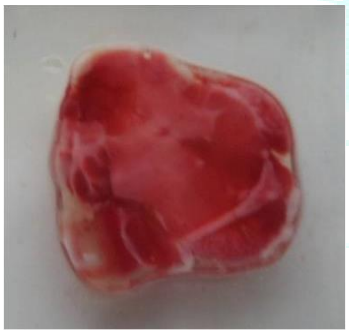
Slide 1: Sham group



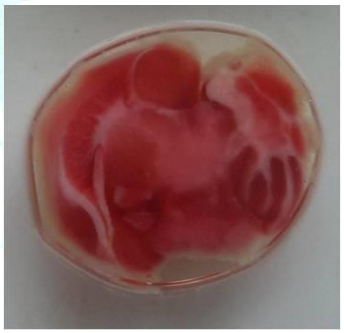
Slide 2: I/R group



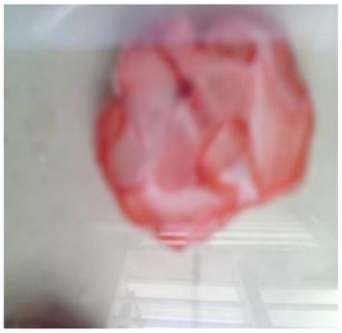
Slide 3: IPC Group

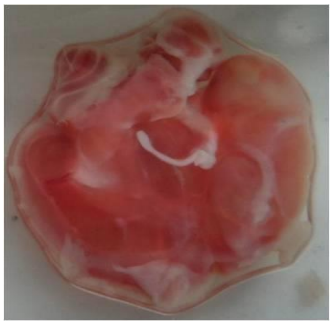


Slide 4: Vehicle treated IPC group



Slide 5: JTC treated group





Slide 6: Glibenclamide treated IP group **Slide 7:** Glibenclamide plus JTC-801 treated IPC group

Figure 1: Effect of treatment/procedure on ischemia and reperfusion induced cerebral infarct size

Effect of ischemic preconditioning and/or treatment on impairment of memory

Global ischemia of 17 minutes followed by reperfusion of 24 hr significantly cause impairment of memory in terms of reduced time spend in target quadrant (Q4) in search of missing platform as compared to sham control group. Three episodes of IPC significantly reduced I/R induced decrease the memory in terms of time spend in target quadrant (Q4) in search of missing platform (Figure 3, Table 1).

Administration of vehicle 30 min prior to three episodes of IPC could not affect protective effect of IPC on memory in terms of time spend in target quadrant (Q4) in search of missing platform. Administration of JTC-801 (1mg/kg, *i.p.)* 30 minutes before the conduction of three episodes of IPC significantly abolished the restoration of IPC induced protective effect of memory noted in terms of time spend in target quadrant (Q4) in search of missing platform. Administration of Glibenclamide (1mg/kg, *i.p.*) 60 minutes before conduction of three episodes of IPC significantly abolished the restoration of IPC induced protective effect on memory noted in terms of time spend in target quadrant in search of missing platform (Q 4). Pretreatment of JTC-801 and Glibenclamide showed a significant ($P<0.05$) loss in memory as by decreasing the time spend in target quadrant (Q4) compare to the sham control group (Figure 3, Table 1).

Administration of JTC-801 (1mg/kg, *i.p.)* 30 minutes before and Glibenclamide (1mg/kg *i.p.*) 60 minutes before the conduction of three episodes of IPC were unable to produce any additional effect on memory noted in terms of time spend in target quadrant (Q 4) in search of missing platform during retrieval trial (Figure 2, 3, Table 1).

Table 1: Involvement of Opioid like receptor1 (ORL1) receptor in ischemic preconditioning induced protection in rat brain.

Group	% Infarct Size	Escape Latency Time (In %)		Time Spend In Target Quadrant (TSTQ in %)
		Day 1	Day 4	
Sham control	8.75%	65.68	26.95	56.20
Ischemia reperfusion (I/R)	66.07%	67.85	28.02	24.29
Ischemic preconditioning (IPC)	27.49%	59.85	28.45	50.32
IPC+ vehicle treated	34.00%	70.30	32.60	43.62
IPC+ JTC-801	52.06%	58.16	29.70	27.95
IPC+ Glibenclamide	46.51%	67.32	30.40	30.60
IPC+JTC-801+Glibenclamide	58.57%	63.80	25.21	25.66

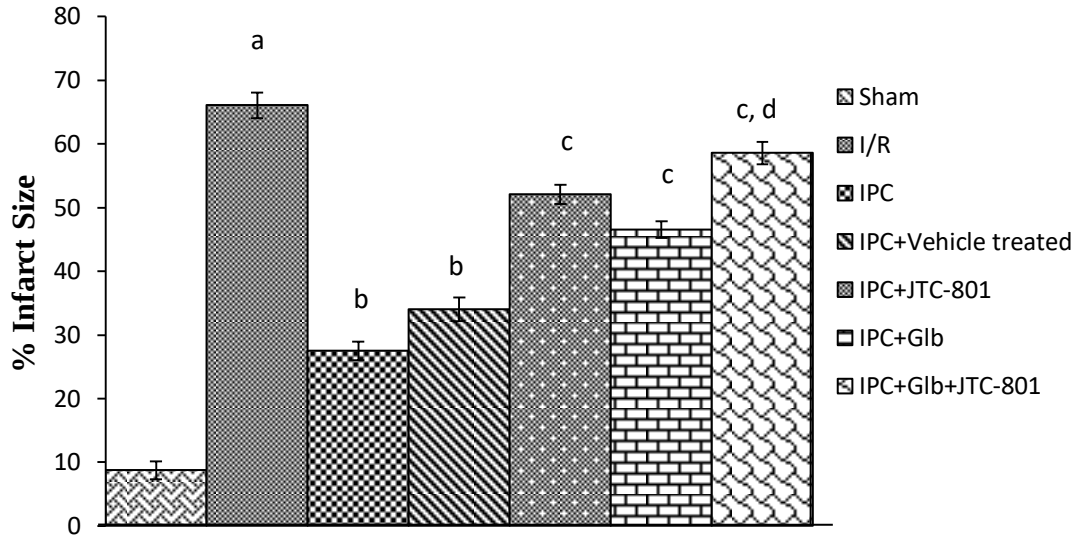


Figure 2: Effect of ischemic preconditioning and interventions on ischemia reperfusion-induced cerebral infarct size. Values are the mean ± SD. a=p< 0.05 vs. Sham control. b =p< 0.05 vs. I/R control. c=p<0.05 vs. IPC. d = p< 0.05 vs. IPC+Glibenclamide treated.

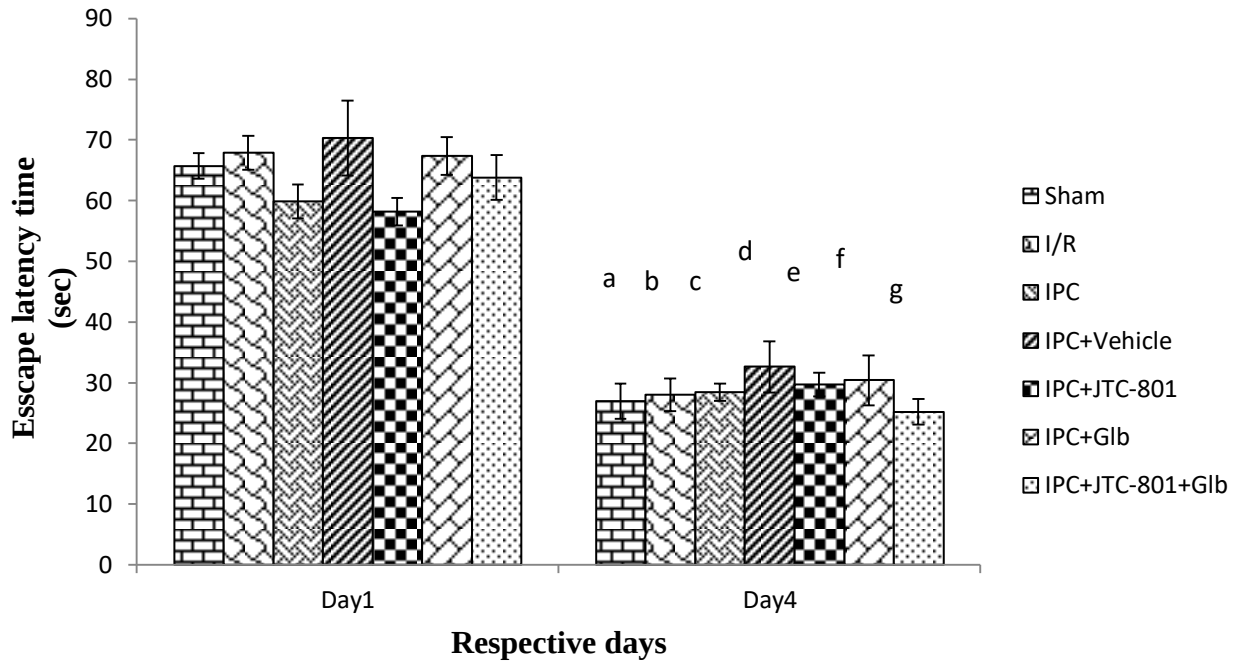


Fig 3: Morris water maze escape latency time of day 1and day 4. IPC-Ischemic preconditioning, I/R-Ischemia reperfusion, Glb-Glebenclamide, Values are expressed as mean ± SD. a =P< 0.05 *vs*. day1 ELT in sham; b =p< 0.05 *vs.* day 1 ELT in I/R; c =p< 0.05 *vs*. day 1 ELT in IPC; d =P< 0.05 *vs*. day 1 ELT in IPC+Vehicle treated; e=p<0.05 *vs.* day ELT in IPC+JTC-801 treated; f =P<0.05 *vs.* day 1 ELT in IPC+Glbtreated; g=p<0.05 *vs.* day 1 ELT in IPC+JTC-801+Glb treated.

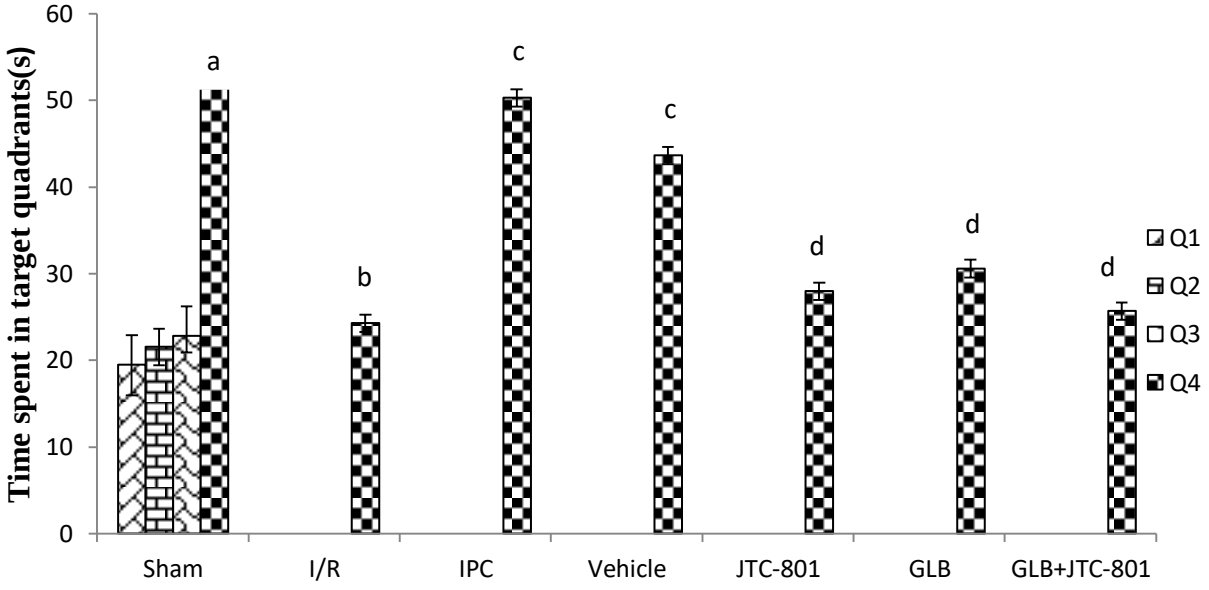


Figure 4: Effect of ischemic preconditioning and interventions on ischemia reperfusion-induced decrease in time spent in target quadrant (TSTQ) as assessed using the Morris water maze. IPC- Ischemic preconditioning; I/R-Ischemia reperfusion; Glb-Glibenclamide. Values are the mean ± SD. a=p< 0.05 *vs.* time spent in other quadrant i.e. Q1, Q2, Q3 in sham group; b =p< 0.05 *vs.* time spent in target quadrant i.e. Q-4 in sham group; c =p< 0.05 *vs*. time spent in target quadrant in I/R group; d =p< 0.05 *vs*. time spent in target quadrant in the ischemic preconditioning (IPC) group.

DISCUSSION

Memory is ability to program, store, retain and recollect information with past experiences in the brain. It can be thought of general terms as the use of past experience to affect or influence present behavior. In present study, wistar albino rats have been used because they are easily available and lower in cost and the global cerebral I/R model employed to report to simulate the clinical situation of cerebral ischemia (Rehni et al., 2008; Jenkins et al., 1981). Global cerebral ischemia is given by 17 min of occlusion followed by reperfusion for 24h. In this study, global cerebral ischemia reperfusion produced a significant rise in infarct size and induced impairment of learning and memory (Rehni et al., 2008; Rehni et al., 2011; Bochelen et al., 1999). In

previous studies it was reported that ischemic preconditioning prevent the ischemia and reperfusion induced cerebral infarct size and impairment of memory (Rehni et al., 2008; Rehni et al., 2011).Therefore in the present investigation we employed Morris water maze test for memory. Significant increment in infarct size and impairment of learning and memory was produced by global cerebral ischemia reperfusion and findings are in line with earlier reports (Rehni et al., 2008; Bochelen et al., 1999). Moreover ischemic preconditioning was observed to prevent ischemia and reperfusion induced cerebral infarct size and impairment of memory which is in concordance with earlier conclusions (Rehni et al., 2008; Rehni et al., 2011; Bochelen et al., 1999; Dunham et al., 1957)

Administration of JTC-801 selective ORL1 receptor antagonist markedly reduced time spent in target quadrant (Q4) in search of missing platform during retrieval trial which reflecting impairment of learning and memory and attenuated the IPC induced decrease in infarct size. Therefore based on the present experimental data, it may be suggested that the beneficial effects of ischemic-preconditioning in global cerebral ischemia-reperfusion induced neuronal injury may be mediated through ORL1 receptor. However the absence of analytical estimation of ORL1 receptor in preconditioned brain as well as in ORL1 inhibited brain is a limitation in the present study and such investigations using histochemical studies may ascertain them intracellular details about the potential role of ORL1 receptor in ischemic preconditioning of brain. Moreover, a detailed study of complete pathway following ORL1 receptor activation that leads to the ischemic preconditioning still requires an exhaustive experimental evaluation. Nevertheless, further studies are needed to confirm the biochemical system that might be involved into the protection served by ischemic preconditioning of brain. KATP channels have been reported to produce neuroprotection (Sun et al., 2010) and Glibenclamide is reported to block KATP channels (Dubey et al., 1999).

In the present study Glibenclamide has attenuated Ischemic preconditioning (IPC) induced cerebral injury, but glibenclamide cause less damage as compare to co administration of both drugs noted in terms of infarct size. Co administration of JTC-801 and Glibenclamide produced detriment additive effect on IPC induced neuroprotection as compare to individual administration of both drugs. Nociceptin on binding to ORL1 receptor shows arteriodilator response by activation of cAMP/cGMP and subsequent opening of KATP (Armstead et al., 1999) which shows neuroprotection in ischemic injury (Suzuki et al., 200). Therefore it may be probable to suggest that neuroprotective effect of IPC may be mediated through up regulation of ORL1 receptor and subsequently opening of KATP channels.

SUMMERY AND CONCLUSION

The present study was designed to investigate the possible involvement of ORL1 receptor in ischemic brain preconditioning in rat brain. Three episodes each comprising 1 min of ischemia by occlusion of carotid artery and 1 min of reperfusion were employed in the present study to produce ischemic brain preconditioning. Infract size was measured to access the effect of ischemic brain preconditioning on ischemia reperfusion induced cerebral injury. Many salient features may be summarized on the basis of results obtained from this study like, firstly three episode of ischemic preconditioning was observed to prevent ischemia and reperfusion induced cerebral infarct size and impairment of memory measured in terms of infract size and time spent in target quadrant (Q4), secondly protective effect of ischemic brain preconditioning was attenuated by JTC-801 (1mg/kg, *i.p.),* an ORL1 receptor antagonist which suggest the involvement of ORL1 receptor induced cerebral protection. Third one is Glibenclamide (1mg/kg, *i.p.*), KATP channel blocker treatment significantly attenuate the cerebroprotective effect of ischemic brain preconditioning, is suggests that cerebroprotective effect of ischemic brain preconditioning is mediated through KATP channels and finally combination of JTC-801 and Glibenclamide produce detrimental additive effect on IPC induced cerebral protection as compared to individual drug. Therefore it may be suggested that the cerbroprotective effect of ischemic brain preconditioning may be mediated through ORL1 receptor and opening of KATP channels.

It may also be concluded that ischemic brain preconditioning induced cerebral protection may be mediated in part by ORL1 receptor and opening of KATP channels.

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REFERENCES

1. Akirav I, Sandi C, Richter-Levin G. Differential activation of hippocampus and amygdala following spatial learning under stress. Eur J Neurosci 2001; 14:719-25.
2. Armstead WM. Age-dependent impairement of K(ATP) channel function following brain injury. J Neurotrauma 1999;16:391-402.
3. Armstead WM. Superoxide generation links protein kinase C activation to impaired ATP- sensitive K+ channel function after brain injury. Stroke 1999; 30:153-59.
4. Bochelen D, Rudin M, Sauter A. Calcineurin inhibitors FK506 and SDZ ASM 981 alleviate the outcome of focal cerebral ischemic/reperfusion injury. J Pharmacol Exp Ther 1999; 288:653-59.
5. Dubey H, Singh A,Patole AM, Tenpe CR, Ghule BV. Allicin, a SUR 2 opener: possible mechanism for the treatment of diabetic hypertension in rats. Revista Brasileira De Farmacognosia (Brazilian Journal of Pharmacognosy) 2012; 22(5):1053-59.
6. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc (Baltimore) 1957; 46:208-9.
7. Fagan SC, Hess DC. Stroke. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey ML. Pharmacotherapy-A Pathophysiologic Approach. McGraw Hill Medical Publishing Division; 2005; 6:415-27.
8. Gidday JM. Cerebral preconditioning and ischaemic tolerance. Nature Reviews Neuroscience 2006; 7(6);437-48.
9. Guo Z, Yao T, Wang J, Ding J. Acute myocardial ischemia upregulated Nociceptin/Orphanin FQ in dorsal root of ganglion and spinal cord of rats. Neuroscience Letters 2008; 433:274-78.
10. Himori N, Wantanabe H, Akaike N, Kurasawa M, Itoh J, Tanaka Y. Cerebral ischemia model with conscious mice. Involvement of NMDA receptor activation and derangement of learning and memory ability. J Pharmacol Method 1990; 23(4):311-27.
11. Jenkins LW, Povlishock JT, Lewelt W, Miller JD, Becker DP. The role of postischemic recirculation in the development of ischemic neuronal injury following complete cerebral ischemia. Acta Neuropathol 1981; 55(3):205-20.
12. Kushwaha S, Malpani A, Aswar UM, Bodhankar SL, Malpani A, Shivakumar SI. Effect of different anesthetic agents on cardiovascular parameters in male wistar rats. Research Journal of pharmaceutical Biological and Chemical Sciences 2011; 2(2):685-90.

13. Lavania M, Yadav HN, Semwal BC. Possible involvement of Opioid Receptor Like (ORL1) receptor in Remote Aortic Preconditioning Induced Cardioprotection in Rat Heart. M Pharm thesis. Institute of Pharmaceutical Research, G L A University, Mathura, Uttar Pradesh, India 2013.
14. Malinowska B, Godlewski G, Schlicker E. Function of Nociceptin and Opioid OP 4 receptors in the regulation of the cardiovascular system. J Physiol Pharmacol 2002; 53(3):301-24.
15. McDonald RJ, White NM. Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. Behav Neural Biol 1994; 61(3):260-270.
16. Mollereau C, Mouledous L.Tissue distribution of the opioid receptor-like (ORL1) receptor. Peptides 2000; 21(7):907-17.
17. Mollereau C, Parmentier M, Mailleux P, Butour JL.,Moisand C, Chalon P, Caput D, Vassart G, Meunier JC. ORL1, a novel member of the opioid receptor family, cloning, functional expression and localization. FEBS Lett 1994; 341(1):33-8.
18. Morris RGM. Development of a water maze producer for studying spatial learning in the rats. J Neurosci Methods1984; 11(1):47-60.
19. Murry, CE, Jennings RB, Reimer KA. Preconditioning with ischemia: A delay of lethal injury in ischemic myocardium. Circulation 1986; 74(5):1124-36.
20. Rawls SM, Schroeder JA, Ding Z, Rodriguez T, Zaveri N. NOP receptor antagonist, JTC 801, blocks cannabinoid-evoked hypothermia in rats. Neuropeptides 2007; 41:239-47.
21. Rehni AK, Singh TG, Jaggi AS, Singh N. Pharmacological preconditioning of the brain: a possible interplay between opioid and calcitonin gene related peptide transduction systems. Pharmacol Rep 2008; 60:904-13.
22. Rehni, Ashish K., et al. Involvement of src-kinase activation in ischemic preconditioning induced protection of mouse brain. Life sciences 2011; 88.19:825-29.
23. Salis MB, Emanueli C, Milia AF, Guerrini R, Madeddu P.Studies of the Cardiovascular effect of nociceptin and related peptides. Peptides 2000; 21(7):985-93.
24. Sharma B, Singh N, Singh M and Jaggi AS. Exploration of HIV protease inhibitor indinavir as a memory restorative agent in experimental dementia, Pharmacol Biochem Behav 2008; 89(4):535-45.
25. Sims NR, Muyderman H. Mitochondria, oxidative metabolism and cell death in stroke. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 2009; 1802(1):80-91.
26. Sun XL, Hu G. ATP-sensitive potassium channels: a promising target for protecting neurovascular unit function in stroke. Clin Exp Pharmacol Physiol 2010; 37(2):243-52.
27. Suzuki M, Saito T, Tamagavoa M, Miki J, Seino S, Naka H. Cardioprotective effect of Diazoxide is mediated by activation of Sarcolemmal but not mitochondrial ATP- sensitive potassium channels in mice. Circulation 2003; 107;682-85.
28. Tomai F, CreanF, Chiariello L, Gioffre PA. Ischemic Preconditioning in Human: Models, Mediators and Clinical Relevance. Circulation 1999; 100:559-63.