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Pharmacological preconditioning of the brain: a possible interplay between opioid and calcitonin gene related peptide transduction systems

Ashish K. Rehni[2], Thakur G. Singh[1], Amteshwar S. Jaggi[1], Nirmal Singh[1]

[1]Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala-147002, Punjab, India

[2]Chitkara College of Pharmacy, Patiala-Chandigarh highway, Rajpura, Patiala-140041 Punjab, India

Correspondence: Nirmal Singh, e-mail: nirmal_puru@rediffmail.com, ashishkrehni@gmail.com

Abstract:
The present study has been undertaken to investigate the possible link between calcitonin gene related peptide (CGRP) and opioid receptor transduction systems in the neuroprotective mechanism of pharmacological preconditioning. Occlusion of the bilateral carotid artery for 17 min, followed by reperfusion for 24 h, was employed to produce ischemia and reperfusion (I/R) induced cerebral injury in mice. Cerebral infarct size was measured by using triphenyltetrazolium chloride staining. Memory was assessed using the Morris water maze (MWM) test. Degree of motor incoordination was evaluated using the inclined beam walk test, rota-rod test, and lateral push test. Morphine (8 mg/kg, *ip*), an opioid agonist, and capsaicin (0.1 mg/kg, *iv*), a CGRP releasing agent, were administered 24 h before surgery to separate groups of animals to induce pharmacological preconditioning. Bilateral carotid artery occlusion, followed by reperfusion, produced a significant increase in the cerebral infarct size and impaired memory as well as motor coordination. Morphine and capsaicin treatment produced both a significant decrease in the cerebral infarct size and a reversal of I/R-induced impairment of memory and motor-coordination. Morphine-induced (8 mg/kg, *ip*) neuroprotective effects were completely decreased by sumatriptan (8 mg/kg, *ip*, a CGRP release inhibitor) administered 1 h before and 6 h and 12 h after morphine administration. Capsaicin-induced neuroprotection was decreased by naloxone (5 mg/kg, *ip*, an opioid antagonist) administered 1 h before and 6 h and 12 h after capsaicin administration. These findings indicate that the transduction systems mediating morphine- and capsaicin-induced pharmacological preconditioning in brain are possibly interlinked with one another.

Key words:
preconditioning, morphine, capsaicin, CGRP, opioid, neuroprotection

Introduction

Ischemic stroke is a syndrome characterized by the rapid onset of neurological injury due to interruption of blood flow to the brain [2]. Although mortality from ischemic stroke has declined over the last decade, it still remains the third leading cause of death, as only limited therapeutic strategies exist. Researchers have also attempted to develop processes that would salvage the ischemic brain from the widespread neuronal damage. Ischemic preconditioning (IPC) is a potent protective strategy introduced by Murray et al. [34] for the ischemic myocardium, which was later applied by Kitagawa et al. to the ischemic neuronal injury [24]. IPC has been demonstrated in other organ systems, including skeletal muscle [31], spinal cord [30], kidney [26], intestine [12] and liver [15]. Subse-

quently, many other forms of preconditioning have also been shown to be beneficial to the ischemic brain, such as inhalational and thermal preconditioning [25, 48]. Furthermore, the neuroprotective manifestations of the phenomena of remote IPC and ischemic postconditioning have also been put forth [39–41]. A detailed mechanistic study of these phenomena has indicated the possibility of pharmacologically activating certain biochemical transduction systems, leading to the appearance of a preconditioning-like protective effect that lasts beyond the agent's elimination, an occurrence commonly referred to as pharmacological preconditioning [42]. Following the realization that various molecular mediators caused IPC, researchers have developed certain agents (or the agonists of their respective receptors) that induce an equally effective form of neuroprotection, particularly adenosine, α-adrenergic, bradykinin, calcitonin gene related peptide (CGRP), opioids, free radicals, nitric oxide, and calcium [7, 13, 28, 37, 44, 45]. Additionally, direct activation of certain intracellular signaling pathways, such as protein kinase C, tyrosine kinase, and mitogen activating protein kinase pathways, and also the direct stimulation of related end effectors and general processes, such as metabolism, protein synthesis, K-ATP channels, Na-K pump, and the cytoskeleton have also demonstrated effectiveness [8, 14, 16, 35]. The main advantage of pharmacological preconditioning over IPC-like interventions is its added clinical feasibility [49]. Both opioid and CGRP receptor activation have been shown to produce a characteristic preconditioning-like ameliorative effect on both the ischemic myocardium and the ischemic brain [16, 32–35]. In our previous study, we reported that the protective effect of remote preconditioning on the ischemic brain is mediated through the endogenous release of opioids and CGRP, with consequent activation of their respective receptors [41]. However, it is not currently clear whether the preconditioning-induced neuroprotection involves the activation of individual opioid or CGRP receptors or if there is a link between these two transduction systems; i.e., does the neuroprotection involve interplay between these two receptors? Moreover, alterations in the opioidergic system have been reported to play a role in mediating the molecular mechanisms underlying the neurochemical responses of brain cells to cerebral ischemia [5]. Activation of the μ opioid receptor has been shown to modulate the ischemic cell change occurring in the damaged central nervous system [25]. The δ and κ opioid receptors have also been proposed to be involved in ischemic neuronal damage, but their role is still controversial, and conclusive data have yet to be obtained [22]. The present study has been undertaken to investigate the effect of morphine- (an opioid agonist) and capsaicin- (a CGRP releasing agent) induced pharmacological preconditioning on ischemic brain injury and to find any possible link between the transduction systems of pharmacological preconditioning-induced neuroprotection elicited by CGRP and opioid receptor activation.

Materials and Methods

Drugs and chemicals

Morphine sulfate (Jackson Laboratories, Amritsar, India), naloxone (Samarth Pharma. Pvt. Ltd., Mumbai, India), capsaicin (Sigma Aldrich Chemical Pvt. Ltd., St Louis, USA), sumatriptan (Panacea Biotech, New Delhi, India), and chloral hydrate (Riedel-deHaen, Germany) were dissolved in normal saline. All other chemicals used in the present study were of analytical grade. All drug solutions were freshly prepared before use.

Ischemia-reperfusion induced cerebral injury

Swiss albino mice of either sex weighing 25 ± 2 g, maintained on a standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water, were employed in the present study. They were housed in the departmental animal house and were exposed to a 12 h cycle of light and dark. The experiments were conducted in a semi-soundproof laboratory. The experimental protocol was approved by the institutional animal ethics committee, and care of the animals was carried out as stated in the guidelines of the 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).

Mice were anesthetized using chloral hydrate (400 mg/kg, *ip*). A midline ventral incision was made in the neck to expose the left and right common carotid arteries, which were isolated from the surround-

ing tissue and vagus nerve. A cotton thread was passed below each of the carotid arteries. Global cerebral ischemia was induced by occluding the carotid arteries. After 17 min of global cerebral ischemia, reperfusion was allowed for 24 h, and the incision was sutured back in layers [20]. The sutured area was cleaned with 70% ethanol and then sprayed with antiseptic dusting powder. The animals were shifted individually to their home cage and were allowed to recover.

A single dose of the agonist drugs, morphine and capsaicin, was given separately to induce pharmacological (drug-induced) preconditioning. The pharmacological preconditioning was induced 24 h prior to the global cerebral ischemia.

Experimental protocol

In total, six groups were employed in the experimental protocol (Fig. 1), and each group consisted of 7 animals. In various animal models of brain ischemia and human stroke, male individuals showed a predominance in suffering from greater amount of neuronal damage as compared to their female counterparts, particularly in terms of larger lesion size, and higher mortality. Clinically, the incidence of stroke is higher in men than in women and is rare in women during the reproductive young age, unless it is caused by subarachnoid hemorrhage. Furthermore, there are some reports on gender specific effects of opioid receptor agonists/antagonists in response to ischemia [25, 50]. Therefore, to minimize variability and to ensure reproducibility, special care was taken that animals were homogenously distributed among the various groups with respect to age and sex.

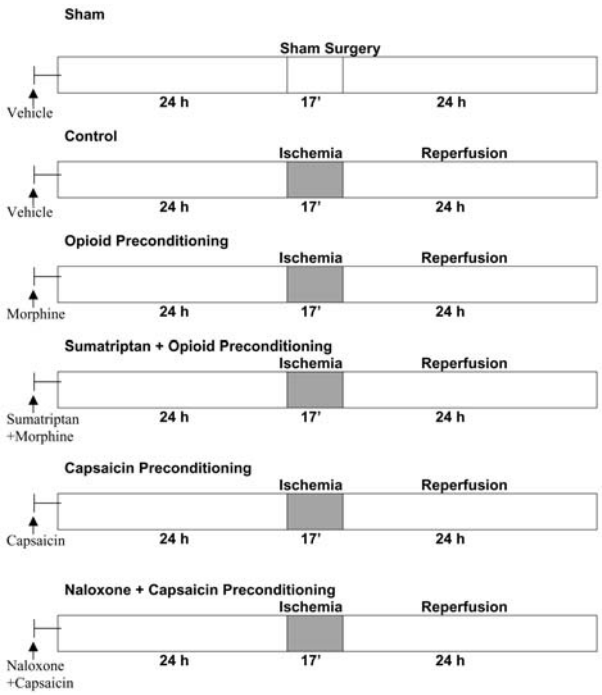

Fig. 1. Schematic representation of the experimental protocol

Sham

Group I (Sham group): each mouse was subjected to surgical procedure, carotid arteries were isolated, and a thread was passed below them, but the arteries were not occluded. After 17 min, threads were removed, and the animal was sutured and allowed to recover for 24 h.

Control

Group II (Control group): each mouse was subjected to 17 min of global cerebral ischemia, followed by reperfusion for 24 h.

Morphine/+ sumatriptan treatment groups

Group III (morphine preconditioning control group): morphine (8 mg/kg, *ip*) was administered 24 h prior to global cerebral ischemia and was followed by 17 min of global cerebral ischemia and 24 h of reperfusion in mice.

Group IV (sumatriptan + morphine preconditioning group): sumatriptan (8 mg/kg, *ip*), a CGRP release inhibitor, was administrated 1 h before and 6 h and 12 h following morphine administration. The remainder of the procedure was the same as described for group III.

Capsaicin/+ naloxone treatment groups

Group V (capsaicin preconditioning group): capsaicin (0.1 mg/kg, *iv*) was administered 24 h prior to global cerebral ischemia and was followed by 17 min of global cerebral ischemia and 24 h of reperfusion in mice.

Group VI (naloxone + capsaicin preconditioning group): naloxone (5 mg/kg, *ip*), an opioid receptor antagonist, was administrated 1 h before and 6 h and 12 h following capsaicin administration. The remainder of the procedure was the same as described for group V.

Assessment of cerebral infarct size

At the end of the 24 h of reperfusion after global cerebral ischemia, animals were sacrificed by spinal dislocation, and the brain was removed and placed immediately in ice cold saline for 10 min. Brain samples were then sliced into uniform coronal sections of about 1 mm thickness. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) at 37°C in 0.2 M Tris buffer (pH 7.4) for 20 min [4]. 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 a glass plate. A transparent plastic grid with 100 squares of 1 cm^2 was placed over it. The average area of each brain slice was calculated by counting the number of squares on either side. Similarly, the number of squares falling over non-stained dull yellow areas was also counted. Infarcted area was expressed as a percentage of total brain volume. Whole brain slices were weighed. The infarcted dull yellow portion was dissected out and weighed. Infarct size was expressed as percentage of total wet weight of the brain.

Evaluation of memory using the Morris water maze

The Morris water maze (MWM) test was employed to assess memory of the animals [33]. The MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1°C). The water was made opaque with non-toxic, white-colored dye. The pool was divided into four equal hypothetical quadrants with the help of two threads, fixed at a right angle to each other on the rim of the pool. A submerged platform (10 cm^2) was painted white and placed in target quadrant, 1 cm below the surface of the water so as to provide an escape area. The position of platform was unaltered throughout the training session.

Acquisition trial

Each mouse was subjected to four trials per day. A rest period of 5 min was allowed between each trial. Four trials per day were repeated for four consecutive days. The starting position to conduct the four training trials was changed on each day as described below, and quadrant Q4 was maintained as the target quadrant in all acquisition trials.

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

Escape latency time (ELT) to locate the hidden platform in the water maze was noted, and the day 4 ELT served as an index of acquisition or learning.

After recording the day 4 ELT, the animal was subjected to the surgical procedure and then subjected to a day 5 retrieval test in the MWM.

Retrieval trial

On fifth day, the platform was removed. Each mouse was placed in water maze and allowed to explore the maze for 120 s. Each animal was subjected to four such trials, and each trial was started from different quadrant. Mean time spent in all three quadrants (Q1, Q2, and Q3) was recorded, and the time spent in the target quadrant (Q4) in search of the missing platform was also noted, which served as an index of retrieval. The experimenter always stood at the same position. For the total duration of the study, care was taken to not disturb the relative location of the water maze with respect to other objects in the laboratory, which could be serving as prominent visual clues. All the trials were completed between 10.00 to 16.00 h.

Evaluation of motor coordination using the rota-rod test

The rota-rod test has been used to evaluate motor coordination by testing the ability of mice to remain on a revolving rod [9]. 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 5 min. Each mouse was given five trials before the actual reading was taken. The animals able to stay on the revolving rod for a period of 5 min before the surgical procedure were selected, and the test was again performed after 17 min of global cerebral ischemia followed by 24 h of reperfusion.

Inclined beam walking test

The inclined beam walking test was employed to evaluate fore and hind limb motor coordination [10]. Each animal was individually placed on a metallic bar 55 cm long and 1.5 cm wide, inclined at an angle of 60° from the ground. The motor performance of the mouse was rated on a scale ranging from 0 to 4. A grade of 0 was assigned to an animal that could readily traverse the beam, grade 1 was given to an animal demonstrating mild impairment, grade 2 was assigned to an animal demonstrating moderate impairment, grade 3 was given to an animal demonstrating severe impairment, and grade 4 was assigned to an animal completely unable to walk on the beam. The inclined beam walking test was performed before global cerebral ischemia and 12 h and 24 h after global cerebral I/R.

Lateral push test

Motor coordination was also evaluated by observing the percentage of mice showing resistance to a lateral push [3]. A mouse was placed on a rough surface to provide firm grip and evaluated for resistance to a lateral push from either side of the shoulder. The test was performed before global cerebral ischemia and 12 h and 24 h after global cerebral ischemia and reperfusion. Mice with increased or decreased resistance to a lateral push after global ischemia were assigned + or – scores respectively.

Statistical analysis

The results were expressed as the mean ± standard error of means (SEM). Statistical analysis for all the results was done using a one-way ANOVA followed by Tukey's multiple range tests as *post-hoc* analysis. The results of the lateral push test were analyzed using chi square test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Effect of pharmacological preconditioning and/or treatments on cerebral infarct size

Global cerebral ischemia of 17 min followed by reperfusion for 24 h (I/R) produced a significant increase in the cerebral infarct size compared to the sham group when measured by both volume and weight methods. Pharmacological preconditioning with morphine (8 mg/kg, *ip*) and capsaicin (0.1 mg/kg, *iv*) administered 24 h prior to ischemia-reperfusion significantly decreased the I/R-induced increase in the cerebral infarct size that was measured by volume and weight methods (Fig. 2).

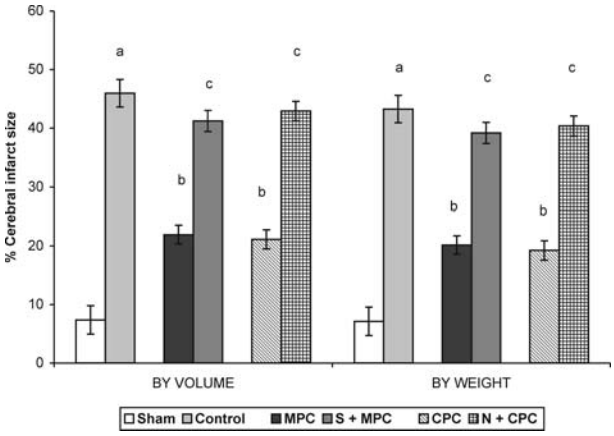

Fig. 2. Effect of pharmacological preconditioning and interventions on ischemia reperfusion-induced cerebral infarct size. MPC – morphine preconditioning; CPC – capsaicin preconditioning; S – sumatriptan; N – naloxone.Values are the mean ± SEM. [a] $p < 0.05$ *vs.* sham group; [b] $p < 0.05$ *vs.* control group; [c] $p < 0.05$ *vs.* respective pharmacological preconditioning (MPC and CPC) group

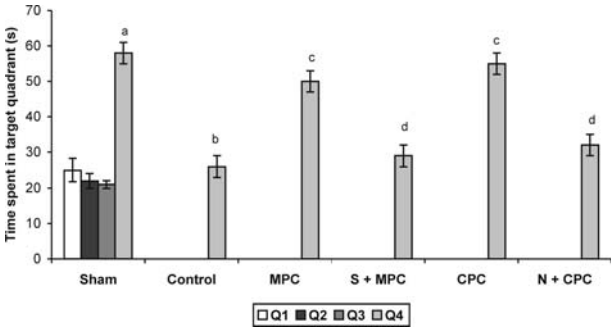

Fig. 3. Effect of pharmacological preconditioning and interventions on ischemia reperfusion-induced decrease in time spent in target quadrant (TSTQ) as assessed using the Morris water maze. MPC – morphine preconditioning; CPC – capsaicin preconditioning; S – sumatriptan; N – naloxone. Values are the mean ± SEM. [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 control group; [d] $p < 0.05$ *vs.* time spent in target quadrant in the respective pharmacological preconditioning (MPC and CPC) group

Pretreatment with sumatriptan significantly decreased the morphine preconditioning-induced decrease in infarct size, whereas naloxone pretreatment decrease the capsaicin preconditioning-induced decrease in cerebral infarct size (Fig. 2).

Effect of pharmacological preconditioning and/or treatments on global cerebral ischemia and reperfusion-induced impairment of memory as assessed using the Morris water maze test

There was a downward trend in escape latency time (ELT) of the animals upon subsequent exposures to the MWM, indicating normal learning abilities. The sham control mice, when subjected to retrieval test on day 5, spent significantly ($p < 0.05$) more time in the target quadrant (Q4) in search of the missing platform as compared to the time spent in other quadrants (Q1, Q2, Q3), reflecting normal memory capacity (Fig. 3). Global cerebral I/R significantly reduced day 5 time spent in the target quadrant (TSTQ), when compared to the sham control animals, reflecting memory impairment. Pharmacological preconditioning induced with morphine (8 mg/kg, *ip*) or capsaicin (0.1 mg/kg, *iv*) produced a significant increase ($p < 0.05$) in the day 5 time spent in the target quadrant, thus attenuating the I/R-induced memory impairment (Fig. 3).

Pretreatment with sumatriptan (8 mg/kg, *ip*) significantly decreased the morphine preconditioning-induced rise in day 5 TSTQ of mice on the MWM. Moreover, naloxone (5 mg/kg, *ip*) pretreatment decreased the capsaicin-induced rise in day 5 TSTQ of the animals (Fig. 3).

Effect of pharmacological preconditioning and/or treatments on global cerebral ischemia/reperfusion-induced impairment of motor performance

Global cerebral I/R-induced impairment of motor performance was assessed by the rota-rod test, inclined beam walking test, and lateral push response.

Effect on fall down time using the rota-rod test

Global cerebral ischemia of 17 min followed by reperfusion for 24 h produced a significant reduction in fall down time measured by rota-rod test, after 24 h of reperfusion, when compared to that of the sham group. Pharmacological preconditioning with morphine (8 mg/kg, *ip*) or capsaicin (0.1 mg/kg, *iv*) administered 24 h prior to ischemic insult decreased the I/R-induced reduction in fall down time. However, pretreatment with sumatriptan and naloxone significantly decreased the rise in fall down time elicited by morphine preconditioning and capsaicin preconditioning, respectively (Fig. 4).

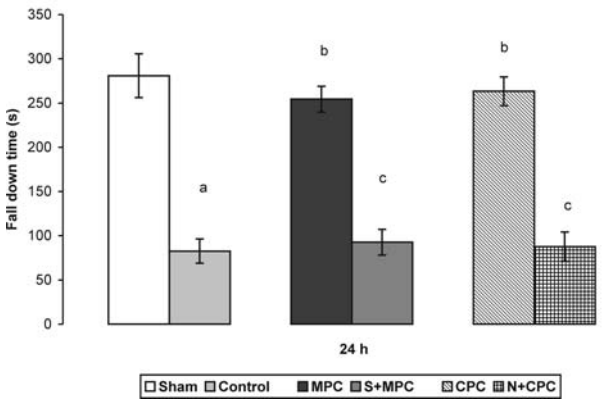

Fig. 4. Effect of pharmacological preconditioning and interventions on ischemia reperfusion-induced changes in motor performance (fall down time) in mice using the rota-rod test. MPC – morphine preconditioning; CPC – capsaicin preconditioning; S – sumatriptan; N – naloxone. Values are the mean ± SEM. [a] $p < 0.05$ *vs.* sham group; [b] $p < 0.05$ *vs.* control group; [c] $p < 0.05$ *vs.* respective pharmacological preconditioning (MPC and CPC) group

Effect on motor incoordination score using the inclined beam walking test

Global cerebral ischemia of 17 min followed by reperfusion for 24 h produced a significant rise in the motor incoordination score, when compared to the sham group as assessed by inclined beam walking test, after 12 h and 24 h of reperfusion. Pharmacological preconditioning with morphine (8 mg/kg, *ip*) or capsaicin (0.1 mg/kg, *iv*) administered 24 h prior to ischemic insult decreased the I/R-induced increase in the motor incoordination score in a significant manner. However, pretreatment with sumatriptan and naloxone significantly decreased the decrease in the motor incoordination score elicited by morphine preconditioning and capsaicin preconditioning, respectively (Fig. 5).

Effect on resistance to lateral push response

Global cerebral ischemia of 17 min followed by reperfusion for 24 h produced a significant decrease in the percentage of mice exhibiting resistance to a lateral

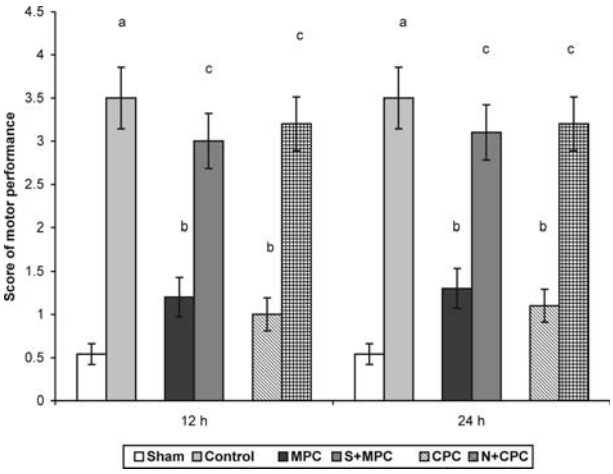

Fig. 5. Effect of pharmacological preconditioning and interventions on ischemia reperfusion-induced changes in motor performance (score of motor performance) in mice using the inclined beam walk test. MPC – morphine preconditioning; CPC – capsaicin preconditioning; S – sumatriptan; N – naloxone. Values are the mean ± SEM. [a] $p < 0.05$ *vs.* sham group; [b] $p < 0.05$ *vs.* control group; [c] $p < 0.05$ *vs.* respective pharmacological preconditioning (MPC and CPC) group

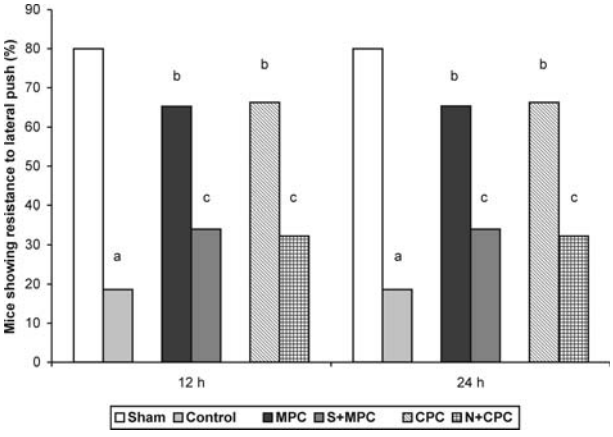

Fig. 6. Effect of pharmacological preconditioning and interventions on ischemia reperfusion-induced changes in motor performance in mice using the lateral push test. Statistical analysis was done using chi-square test. MPC – morphine preconditioning; CPC – capsaicin preconditioning; S – sumatriptan; N – naloxone. Values are percentage of mice demonstrating resistance to lateral push test. [a] $p < 0.05$ *vs.* sham group; [b] $p < 0.05$ *vs.* control group; [c] $p < 0.05$ *vs.* respective pharmacological preconditioning (MPC and CPC) group

push, which was noted after 12 h and 24 h of reperfusion, when compared to the sham group. Pharmacological preconditioning with morphine (8 mg/kg, *ip*) or capsaicin (0.1 mg/kg, *ip*), administered 24 h prior to ischemic insult, decreased the I/R-induced decrease in the percentage of mice demonstrating resistance to a lateral push when compared to the control group. However, pretreatment with sumatriptan and naloxone significantly reversed the decrease in the percentage of mice demonstrating resistance to a lateral push elicited by morphine preconditioning and capsaicin preconditioning, respectively (Fig. 6).

Discussion

The global cerebral I/R model employed in the present study is reported to simulate the clinical situation of cerebral ischemia [1]. Cerebral ischemia has been reported to impair memory because hippocampal neurons are susceptible to the deleterious effects of I/R and the hippocampus is involved in the regulation of memory [23]. Cerebral ischemia has been further documented to impair motor ability as well [43]. Therefore, in the present investigation, we employed the MWM test to assess memory and the rota-rod test, inclined beam walk test, and lateral push test to evaluate motor coordination. In our study, global cerebral I/R produced a significant increase in infarct size and induced impairment of memory, as well as of motor coordination. These findings are in agreement with earlier reports [13, 14, 18, 20].

In the present study, morphine administration 24 h prior to a severe ischemic insult resulted in a considerable reduction in the I/R-induced cerebral injury, when measured in the terms of cerebral infarct size and impairment of memory and motor-coordination, which is consistent with earlier findings [19, 52]. Furthermore, the noted neuroprotective effect of morphine preconditioning was decreased by sumatriptan pretreatment (a CGRP release inhibitor), thus implicating CGRP in the sustained neuroprotection afforded by the atypical non-selective opioid receptor agonist, morphine [17]. In agreement with the observations above, it has also been documented that morphine pretreatment (preconditioning)-induced activation of opioid receptors elicits a systemic release of CGRP, and the consequent activation of the CGRP receptor mediates the cardioprotective effects of morphine preconditioning [51]. Additionally, both opioid and CGRP receptors are widely distributed throughout the central nervous system [29, 38]. Therefore, it may be proposed that following the phase of mor-

phine preconditioning, CGRP may be released from the nerve terminals This release may be causing the activation of a CGRP linked transduction system, resulting in the observed neuroprotection. Such a hypothesis is further supported by the results of some workers, who have shown that extended opioid exposure elicits an increased release of calcitonin gene related peptide in the synaptic region of the dorsal root ganglia, resulting in pronociceptive consequences of prolonged morphine exposure [29].

The results of the present study further demonstrated that capsaicin administration also exerted a pharmacological preconditioning-like protective effect, which is also in agreement with previous reports [41, 47]. Capsaicin has been shown to systemically activate the release of CGRP throughout the vasculature [21]. Furthermore, the activation of CGRP receptors is reported to mediate the protective effects of IPC [47]. Recently, we have reported that remote IPC of the brain also exerted its neuroprotective effects *via* the release of endogenous CGRP and consequent activation of CGRP receptors in the brain [41]. Therefore, the capsaicin-induced pharmacological preconditioning observed in this study also appears to be mediated through the endogenous release of CGRP with subsequent activation of central CGRP receptors. Such a postulation is further supported by a study demonstrating the role of endogenous opioids as mediators of the hypothermic effects of intrathecally administered calcitonin gene-related peptide in mice [46].

Consistent with the hypothesis above, this study further demonstrated that the neuroprotective effect of capsaicin preconditioning was decreased by naloxone. Naloxone is an antagonist of multiple subtypes of opioid receptors, which are abundantly expressed in the central nervous system [51]. It has also been documented that activation of opioid receptors plays a vital role in mediating multiple biochemical events triggered by CGRP [6]. The noted inhibitory effect of naloxone on capsaicin preconditioning suggests a possible implication of opioid receptor activation in the CGRP-mediated neuroprotection. Based on the discussion above, it may be deduced that, while morphine preconditions the brain *via* a CGRP receptor activation-linked mechanism, capsaicin conditions it by an opioid receptor linked pathway. These observations suggest that these two transduction systems involved in the respective pharmacological preconditionings evoked by morphine and capsaicin might actually be linked. There might be a possible interplay between central CGRP and opioid receptors in mediating the neuroprotective effect of the pharmacological preconditioning. Both endogenous opioids, as well as CGRP, have been reported to enhance the release of one another and thus have been used to explain a number of observations [36, 46]. Given the fact that the activity of both receptors is largely based on the intracellular dynamics of calcium, this interplay might be linked to changes in the intracellular concentration of the very important ion, calcium. However, such a hypothesis requires a detailed experimental evaluation before it may be considered by the scientific community.

Furthermore, current data indicate the paradoxical neuroprotective effect of both opioid receptor agonists, as well as antagonists [11, 27]. In the present study, transient opioid receptor activation using a single dose of morphine 24 h prior to the induction of cerebral ischemia was found to elicit a preconditioning-like adaptive response in the brain cells, which lasted even after the supposedly complete excretion of the atypical opioid agonist morphine. However, naloxone at a dose level of 5 mg/kg decreased the protective effect of capsaicin preconditioning, implicating the involvement of opioid receptor activation linked mechanism in the mediating capsaicin preconditioning. At a different dose, naloxone has been shown to exhibit a neuroprotective effect. Our laboratory had reported earlier that naloxone, at the dose of 5 mg/kg, *ip*, did not exert any significant neuroprotective effect on the ischemic mouse brain [41]. Therefore, this dose of naloxone was used in this investigation to study the role of opioid receptors in capsaicin preconditioning, while at the same time avoiding the neuroprotection that is induced by naloxone itself.

Conclusion

It can be concluded that the neuroprotective effect of pharmacological preconditioning induced by morphine or capsaicin involves an interplay between the opioid and CGRP receptor linked signal transduction cascades. However, further studies are required to delineate the various biochemical changes leading to such molecular cross-talk.

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