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Piroxicam-mediated modulatory action of 5-hydroxytryptamine serves as a "brake" on neuronal excitability in ischemic stroke

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Abstract

Our previous studies indicated an increase in extracellular γ-aminobutyric acid (GABA) in rodent's ischemic brain after Piroxicam administration, leading to alleviation of glutamate mediated excitotoxicity through activation of type A GABA receptor (GABAA). This study was to investigate if GABAA activation by Piroxicam affects extracellular 5-hydroxytryptamine or not. High performance liquid chromatography revealed that there was a significant decrease in extracellular 5-hydroxytryptamine release in ischemic cerebral cortex and striatum in Piroxicam pre-treated rat brains. This suggests a probable role of Piroxicam in reducing extracellular 5-hydroxytryptamine release in ischemic cerebral cortex and striatum possibly due to the GABAA activation by Piroxicam.

Key Words: *nerve regeneration; ischemic stroke; serotonin; γ-aminobutyric acid; neuroprotection; piroxicam; cerebral cortex; striatum*

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Introduction

Neurotransmitter imbalance in ischemic stroke leads to devastating consequences. In stroke, γ-aminobutyric acid (GABA) has a regulatory role in glutamate release in the brain regions and helps redress glutamate-mediated excitotoxicity (Bhattacharya et al., 2014). 5-Hydroxytryptamine (5-HT) induces a decrease of glutamate transmission and a parallel increase in GABA transmission particularly in the frontal cortex (Zhou and Hablitz, 1999). Piroxicam-mediated modulatory action of 5-HT may serve as a "brake" on neuronal excitability. Hence we speculate that 5-HT modulation may participate to reduce pathogenesis in stroke. Owing to our past results regarding neuroprotective efficacy of Piroxicam in cerebral ischemia (Bhattacharya et al., 2013) and its role in modulating the exogenous GABA to bring changes in the extracellular release of glutamate (Bhattacharya et al., 2014), in this study, we investigated the effects of Piroxicam on 5-HT level and a probable link of GABA agonism following cerebral ischemia.

Materials and Methods

Animals

Ninety-six male Charles Foster rats, aged 6 weeks, weighing 270 ± 10 g, were used for the experiments. Rats were kept under standard laboratory conditions. These rats were fasted overnight and maintained at a 12-hour light/dark cycle. Then they were divided into blank control (n = 6), middle cerebral artery occlusion (MCAO; n = 6) and MCAO + Piroxicam (n = 84) groups. MCAO was performed according to a previously described method (Bhattacharya et al., 2014). Rats in the MCAO + Piroxicam group were administered Piroxicam (Sigma-Aldrich, St.Louis, MO, USA; 10 mg/kg body weight, i.p.), which was pre-dissolved in normal saline, 30 minutes prior to MCAO (Bhattacharya et al., 2013). Fourteen time points were selected to optimize the baseline in vehicle and drug-treated vehicle (in triplicate). The rats that either did not show significant reduction in cerebral blood flow by 70 % or died during surgery or after surgery before completing the experimental time frame were excluded from the study. The approved standard procedures and the institutional animal ethical committee guidelines of Banaras Hindu University, India were followed throughout the experiments (Approval No. 542/AB/CPCSEA).

Methods

High performance liquid chromatography (HPLC)-electrochemical detection (Waters, Meliford, MA, USA) was performed to quantify extracellular 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels as per a previously described method (Borah and Mohanakumar, 2009). Quantitation of extracellular 5-HT and 5-HIAA in the same cerebral cortex and striatum was performed using the same method. GABA/glutamate study was conducted according to a method described by Bhattacharya et al. (2014). Rats were euthanized by decapitation at different time points as most relevant to significant neurochemical changes. Extracted brains were immediately dissected for harvesting the cerebral cortex

Table 1 Effect of Piroxicam on 5-hydroxytryptamine (5-HT, μg/g of tissue weight) and its derivative 5-hydroxyindoleacetic acid (5-HIAA, μg/g of tissue weight) release in the ischemic rat cerebral cortex and striatum

	5-HT		5-HIAA	
Group	Cortex	Striatum	Cortex	Striatum
Blank control	0.524±0.012	0.425±0.004	0.112±0.017	0.101±0.021
MCAO	0.567±0.001	0.487±0.025	0.149±0.009	0.135±0.006
MCAO+Piroxicam	0.537±0.032	0.462±0.008	0.125±0.045	0.109±0.07

MCAO: Middle cerebral artery occlusion.

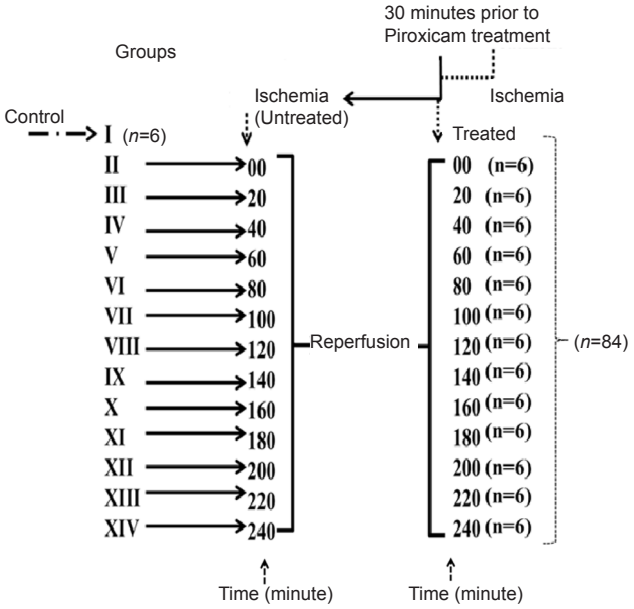

Figure 1 Experimental design.

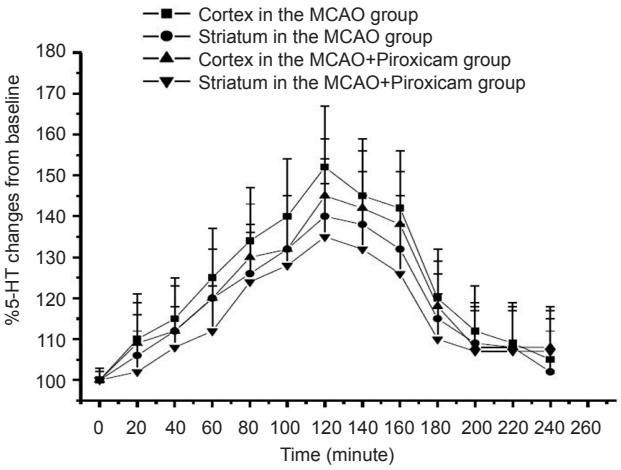

Figure 3 Piroxicam reduced extracellular 5-hydroxytryptamine (5-HT) release in the ischemic rat cortex and striatum.
Data are expressed as the mean ± SD of percentage changes from basal level across the first five samples. MCAO: Middle cerebral artery occlusion.

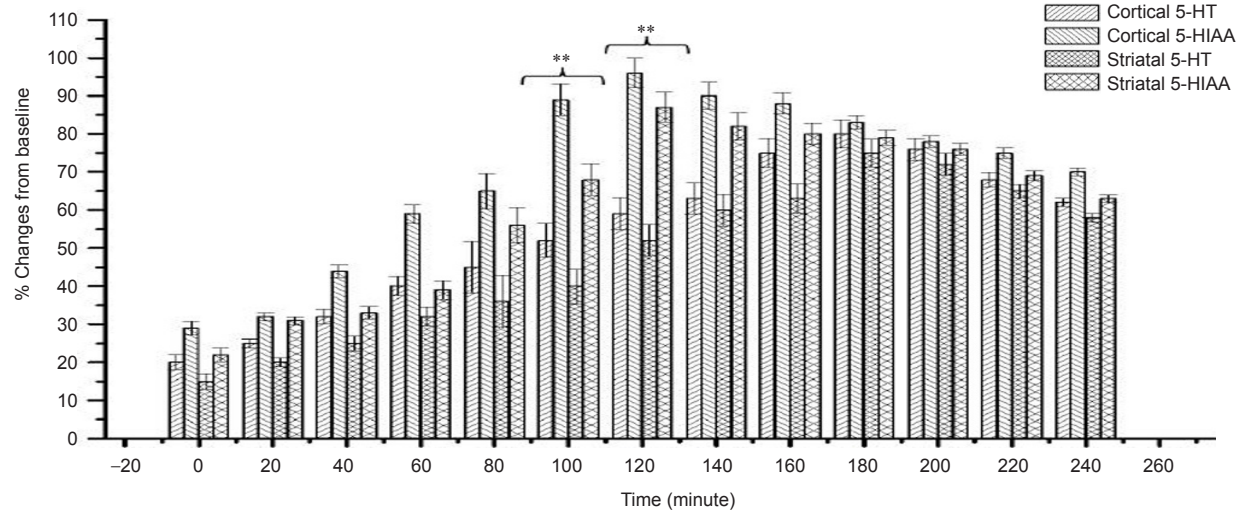

Figure 2 Time optimization of 5-hydroxytryptamine (5-HT) and its derivative 5-hydroxyindoleacetic acid (5-HIAA) release in the ischemic rat cerebral cortex and striatum.
Data are expressed as the mean ± SD of a percentage change from baseline, which is the mean of the first two samples *vs*. each corresponding value in the middle cerebral artery occlusion group. Middle cerebral artery occlusion + Piroxicam group had a significant overall decrease in 5-HT (**$P < 0.01$).

and striatum. Dissected regions were stored at −80°C until the time of analyses. For neurotransmitter assay, an HPLC system equipped with a Rheodyne syringe injector (20 μL) and an electrochemical detector was used for the assays in which the samples were injected directly into a C18 ion pairing column (4.6 mm × 250 mm; pore size 8 nm; particle size 5 μm). The following conditions were used for mobile phase: 8.65 mM heptane sulfonic acid, 0.27 mM ethylenediaminetetraacetic acid (EDTA), 13% acetonitrile, 0.4–0.45% triethylamine and 0.32–0.35% phosphoric acid (v/v). The reaction mixture was prepared with degassed de-ionized water. Flow rate was 0.7 mL/min, and electrodetection was performed at +740 mV for analyses. Data are expressed as the mean ± SD. Significance was analyzed using one-way analysis of variance followed by Tukey's *post hoc* test. A P value of < 0.05 was considered statistically significant. Precise protocol is shown in **Figure 1**.

Results and Discussion

Piroxicam has shown neuroprotective effects in rodent models of ischemic stroke (Bhattacharya et al., 2013). It increases extracellular GABA level in brain regions to alleviate glutamate-mediated excitotoxicity as a compensatory mechanism, possibly by GABAA agonism (Bhattacharya et al., 2014). Our present study investigated changes in extracellular release of 5-HT and its metabolite 5-HIAA in relation to extracellular GABA increase in different rat groups (**Figure 2**). Prior to MCAO, extracellular 5-HT and 5-HIAA levels were 0.949 ± 0.016 μg/g and 0.213 ± 0.038 μg/g respectively. Following MCAO, 5-HT and 5-HIAA levels peaked at 120 minutes (1.054 ± 0.026 μg/g, 0.284 ± 0.015 μg/g). In the MCAO + piroxicam group, 5-HT level had an overall significant decrease (%) (1.338x X/100 = 1.233). In the MCAO + Piroxicam group, 5-HT and 5-HIAA levels peaked at 120 minutes after Piroxicam administration (0.999 ± 0.040 μg/g, 0.234 ± 0.052 μg/g), while at this time point, 5-HT and 5-HIAA levels in the MCAO group were 1.054 ± 0.026 μg/g and 0.284 ± 0.015 μg/g, respectively. That is to say, Piroxicam reduced as much as 92.15 % of released 5-HT and 82.39% of released 5-HIAA (**Figure 2**). Piroxicam administered group had decreased 5-HT release after 120 minutes by as much as 0.537 ± 0.032 *versus* 0.462 ± 0.008 with the peak value of serotonin being only 0.567 ± 0.001 *versus* 0.487 ± 0.025 to that of ischemic group (**Figure 2**), indicating that Piroxicam decreased 5-HT release in ischemic brain.

5-HT plays a determining role in stroke pathophysiology and influences the neurotransmitter system of the brain. Increased 5-HT activity in the brain after stroke is life-threatening. There is evidence that 5-HT induces a decrease of glutamate transmission and a parallel increase in GABA transmission particularly in the frontal cortex (Zhou and Hablitz, 1999). Possible reasons behind this include: a) receptor recruitment on the post-synaptic membrane; b) receptor modulation to promote phosphorylation; c) common signaling pathway of G protein, adenylate cyclase or phospholipase C; and d) effects on ion channels influencing neuronal excitability (Okuhara and Beck, 1994; Premkumar and Gage, 1994; Sodickson and Bean, 1998; Li et al., 1999, 2000; Paolucci et al., 2003).

Our results are in accordance with a previous study (Zhou and Hablitz, 1999) where GABA-mediated inhibitory transmission can strongly be modulated by 5-HT in parallel to 5-HT glutamate interactions.

On the basis of our findings we hypothesized that activation of GABA receptors by Piroxicam might play a role in reducing the harmful action imposed by increased efflux of serotonin in the ischemic brain regions. Results from this study showed that the increase in extracellular GABA from baseline in the cortex and striatum induced by MCAO was greater than that of extracellular 5-HT, but the peak level of GABA after ischemia was only one-third of that of 5-HT.

Therefore, it is reasonable to use Piroxicam to examine the regulatory effects of GABA on 5-HT release. Administration of Piroxicam diminished abnormally elevated 5-HT level induced by cerebral ischemia by as much as 82.39% (**Figure 3**), indicating that GABA probably has an inhibitory effect on 5-HT release during ischemia (**Table 1**). Our results indicate that GABA receptor plays a role in regulating extracellular 5-HT release in rat brain during ischemia. These results suggest that Piroxicam pretreatment provides pre-conditioning effect on combat ischemia induced increase in extracellular glutamate and 5-HT. Results also suggest that Piroxicam serves as a 5-HT and glutamate receptor antagonist and a GABA agonist following cerebral stroke. Detailed studies involving receptor binding and inhibition are underway to draw a concrete conclusion.

Author contributions: *PB and RP designed this study; PB, AKP and SP performed experiments. PB, AKP and RP analyzed the results. All authors approved the final version of this paper.*
Conflicts of interest: *None declared.*

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