

Dopaminergic but Not Glutamatergic Neurotransmission Is Increased in the Striatum after Selective Cyclooxygenase-2 Inhibition in Normal and Hemiparkinsonian Rats

Hadi Fathi Moghaddam³, Mehdi Shafiee Ardestani¹, Mostafa Saffari², Latifeh Navidpour¹, Abbas Shafiee¹ and Arman Rahmim⁴

¹Department of Medicinal Chemistry and Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran,

²Department of Pharmaceutics, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran,

³Department of Physiology, School of Medicine and Physiology Research Center, Jondishapour University of Medical Sciences, Ahwaz, Iran, and ⁴Department of Radiology, Johns Hopkins University, Baltimore, MD, USA

(Received December 12, 2007; Accepted February 6, 2008)

Abstract: In the present work, we studied the effect of the selective cyclooxygenase-2 (COX-2) inhibitors, compound 11 g, celecoxib and selective COX-1 inhibitor SC-560 (intraperitoneally and acutely) on striatal glutamatergic and dopaminergic neurotransmission in normal and substantia nigra pars compacta (SNc)-lesioned rats using the microdialysis technique. We also investigated the effect of acute COX inhibition on the damaged SNc neurons. Our results indicate a significant increase in dopaminergic neurotransmission and a decrease in glutamatergic neurotransmission ($P < 0.05$) only after selective COX-2 inhibition in the striatum of normal and hemiparkinsonian rats. Nonetheless, neither COX-1 nor COX-2 inhibitors showed any improvement in the damaged SNc neurons.

Parkinson's disease is a degenerative neurodopaminergic disease in the nigrostriatal pathway of human beings. Increasing evidence suggests that an inflammatory reaction accompanies the pathological processes, caused by cyclooxygenase-2 (COX-2) seen in many neurodegenerative disorders, including Parkinson's disease. COX-2 is the inducible form of the enzyme COX and in contrast to the other isoform (COX-1) is up-regulated under several stressful conditions such as cerebral damage. It appears to be expressed in dendrites and cell bodies of neurons in several areas of the brain, such as the nigrostriatal pathway, CA-1 hippocampus and amygdala nucleus [1–3]. Normally, COX-2 is expressed in low levels in nigral dopaminergic neurons, but it becomes up-regulated in both patients and in Parkinson's disease models [4,5]. In the central nervous system, COX-2 plays an important role in membrane excitability, synaptic transmission and participates in memory consolidation during rapid eye movement sleep [3–6].

Furthermore, COX-2 inhibitors, such as celecoxib (selective) or aspirin (non-selective), have been shown to provide some neuroprotection or rigidity recovery in Parkinsonian animal models [1,2]. 6-Hydroxy-dopamine (6-OHDA)-induced lesions are the oldest model used to produce Parkinson-like disease in rodents and continue to be the most popular experimental models of Parkinson's disease. This compound does not cross the blood-brain barrier and must be injected directly into the brain by stereotaxic means. 6-OHDA is a prototypic oxidative stress neurotoxin and exhibits a high affinity for

dopaminergic and norepinephrine transporters, and can enter both dopaminergic and noradrenergic neurons [7].

The present work studies assays of neurotransmitter glutamate and dopamine concentrations in the striatum of normal and hemiparkinsonian rats following administration of selective COX-1 or COX-2 inhibitors.

Materials and Methods

Animals. Adult male albino Wistar rats (200–250 g) were used in the study. The animals were housed in groups of six in stainless steel cages, handled daily, and provided food and water *ad libitum*. A 12-hr light:dark cycle was maintained, and the animals were tested during the light cycle. These animal experiments were carried out in accordance with recommendations from the Declaration of Helsinki and the internationally accepted principles for the use of experimental animals. The animals were defined either as normal or Parkinsonian group and each group was divided into seven subgroups (each subgroup contained six rats) who received COX inhibitors or vehicle. Three additional control groups were defined as normal and substantia nigra pars compacta (SNc)-lesioned and probe control.

Drugs and solvents. Celecoxib was purchased from the Razak Laboratory (Pharmaceutical Company, Tehran, Iran) SC-560, and ketamine and xylazine were purchased from Merck (Darmstadt, Germany). Compound 11 g was prepared as previously described [8]. All COX inhibitors dissolved freely in dimethyl sulfoxide (DMSO) 100% and diluted with saline before injection in this manner ketamine and xylazine dissolved in distilled water.

Surgery. Each rat was anaesthetized separately by injection of 75 mg/kg ketamine combined with 8 mg/kg xylazine intraperitoneally. Then, the rats were prepared for surgery and placed in the stereotaxic instrument. A sagittal incision was made in the scalp with a sterile blade, the skin and inferior tissue layers covering the skull were retracted, and a small hole was drilled at the following coordinates:

Author for correspondence: Mehdi Shafiee Ardestani, Department of Medicinal Chemistry and Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (fax +98-021-66929356, e-mail shafieeardestani@razi.tums.ac.ir).

–4.8 mm posterior and –1.6 mm lateral to bregma and 8.2 mm ventral to the surface of the skull for the left SNc according to the atlas. Vehicle (0.2% ascorbic acid) or 6-OHDA (8 µg in 1 µl in 0.2% ascorbic acid) was infused unilaterally through a 26-gauge stainless steel cannula into the SNc region thus creating hemiparkinsonian rats. Then, the skull was exposed and a hole was drilled through it in the area overlying the right striatum, using the following coordinates with respect to the bregma: A/P + 1 mm; M/L + 3 mm, D/V + 6 mm according to the atlas [9]. A guide cannula was lowered into the brain for inserting the microdialysis probe which delivered a modified Ringer's solution through the probe, it was fixed to the cranium and the incision was closed. Surgery was performed using sterile instruments and aseptic conditions.

Microdialysis procedure. Rats were allowed to recover from the surgery for 7–10 days. Microdialysis experiments were performed in normal and SNc-lesioned animals. On the experimental day, a microdialysis probe was inserted into the cannula, and the inputs of the probes were connected to a microperfusion pump, CMA/102 infusion pump (CMA/Microdialysis, Solna, Sweden), which delivered a modified Ringer's solution (147 mM NaCl, 1.2 mM CaCl₂, 2.7 mM KCl, 1.0 mM MgCl₂ and 0.04 mM ascorbic acid) through the probe at a flow rate of 2 µl/min. The Ringer's solution was then infused for 3–3.5 hr before the baseline samples were collected to obtain stable basal extracellular levels of dopamine or glutamate. The microdialysate samples (20 µl) were collected every 20 min. When a stable outflow was shown by four consecutive samples of neurotransmitters, rats were given celecoxib (3.6 mg/kg) and compound 11 g (2.4 mg/kg) which we [8] previously reported as a new type of 1-aryl-5-(4-methylsulfonylphenyl)imidazoles, possessing C-2 alkylthio (SMe or SEt) substituents, which was designed and synthesized and then evaluated as selective COX-2 inhibitor with *in vivo* anti-inflammatory activity. The compound 11 g was the most potent and selective COX-2 inhibitor (COX-2 IC₅₀ = 0.43 µM with no inhibition of COX-1 up to 25 µM) relative to the reference drug celecoxib (COX-2 IC₅₀ = 0.21 µM with no inhibition of COX-1 up to 25 µM) and also showed very good anti-inflammatory activity compared to celecoxib in carrageenan-induced rat paw oedema assay [8] and SC-560 (1.2 mg/kg) as selective COX-1 inhibitor and DMSO as vehicle. The control rats received a saline injection (1 ml/kg) intraperitoneally. The dialysates were collected for 4 hr after the administration of COX-2- or COX-1-selective inhibitors. The stress caused by the intraperitoneal vehicle injection and handling of the rats was not found to alter the extracellular glutamate–dopamine levels. In part of the experiments, when the rats were given drugs or vehicle after four stable consecutive samples, the dialysates were collected for 2.5 hr after the injection. Microdialysate levels of glutamate and dopamine were analysed immediately. After the experiments, the location of the probe was determined histologically on serial coronal sections. Only data obtained from rats with correctly implanted probes were included in the results. All experiments were made with awake, conscious animals. Animals were individually housed for the duration of the experiment in a CMA/120 system (CMA/Microdialysis).

Glutamate and dopamine samples were analysed by reverse-phase high performance liquid chromatography with electrochemical detection [10,11]. Following that, histological evaluations were employed to investigate whether COX-2 or COX-1 inhibition improved the lesion [12]. By preparing the mean ± S.E.M. The average concentrations of four stable samples before drugs or vehicle (DMSO) administration were considered as the basal glutamate and dopamine concentrations. Statistical evaluation of the results was performed by means of one-way ANOVA and Student–Newman–Keuls multiple range test, considering the following significant differences: $P < 0.05$.

Results

The striatal extraneuronal (i.e. microdialysate) concentrations of glutamate in normal and SNc-lesioned rats of all examined

groups before drug–vehicle injection (baseline) were 2.2 ± 0.23 and 2.91 ± 0.38 ng/20 µl, with dopamine concentrations of 3.11 ± 0.49 and 2.1 ± 0.33 pg/20 µl, respectively, for normal and SNc-lesioned rats.

COX-2 inhibitors and doses were observed to modify glutamatergic and dopaminergic neurotransmission in the striatum of hemiparkinsonian rats within the observation period, as depicted in fig. 1A,B. The changes were significant ($P < 0.05$) for glutamate and dopamine concentrations at 40 min. and throughout until 180 min. Comparing with the normal rats that received only saline, COX-2 inhibitors were able to decrease glutamate or increase dopamine to the normal levels and even more than the normal level at or after 60 min.

Statistically, low doses of COX-2 inhibitor administration to normal rats had no significant effect on glutamatergic nor dopaminergic neurotransmission but the high doses produced a significant decrease in glutamatergic neurotransmission and an increase in dopaminergic neurotransmission ($P < 0.05$) within 60–120 min. only (fig. 1C,D). Statistical analysis showed that the high doses of COX-2 inhibitors significantly attenuated glutamatergic neurotransmission in both normal (19.04%, average of 80–120 min.) and hemiparkinsonian rats (25.43%, average of 40–180 min.). At the same time, dopaminergic neurotransmission was enhanced in both normal (13.82%, average of 80–120 min.) and hemiparkinsonian rats (49.52%, average of 20–180 min.) by high doses of COX-2 inhibitors, respectively. It should be noted that the selective COX-1 inhibitor SC-560 had no significant effect on glutamatergic nor dopaminergic neurotransmission in normal and SNc-lesioned rats (data not shown).

Interestingly, histological results show that treatment with either COX-2 or COX-1 inhibitors did not improve the lesion (fig. 2A–D).

Discussion

This study was intended to explore the important role for COX-2 in glutamatergic and dopaminergic neurotransmission in the striatum of normal and hemiparkinsonian rats. Additionally, this study shows that acute COX inhibition does not have any improving effect on damage of SNc neurons. It seems that COX-2 and its metabolites, prostaglandins (PG), have an important role in neurotransmitter release as noted in previous reports [13,14], suggesting that COX-2 causes increased levels of acetylcholine in the brain via production of PGE₂, and increases in expression of cholinergic markers, such as choline acetyltransferase and vesicular acetylcholine transporter protein. It was also noted that prostaglandins have modulatory effects on adrenergic, noradrenergic and glutaminergic transmission, specially PGE₂, and prostaglandin synthesis inhibitors induced increases in the blood pressure via increases in the release of the catecholamine; for example, using large doses of glucocorticoids in human beings may cause insomnia, euphoria and increase the intracranial pressure [13,14]. Additionally, an *in vitro* study evaluated the effects of some non steroidal anti-inflammatory drug (NSAIDs) on cultured primary rat embryonic neurons from rat embryonic

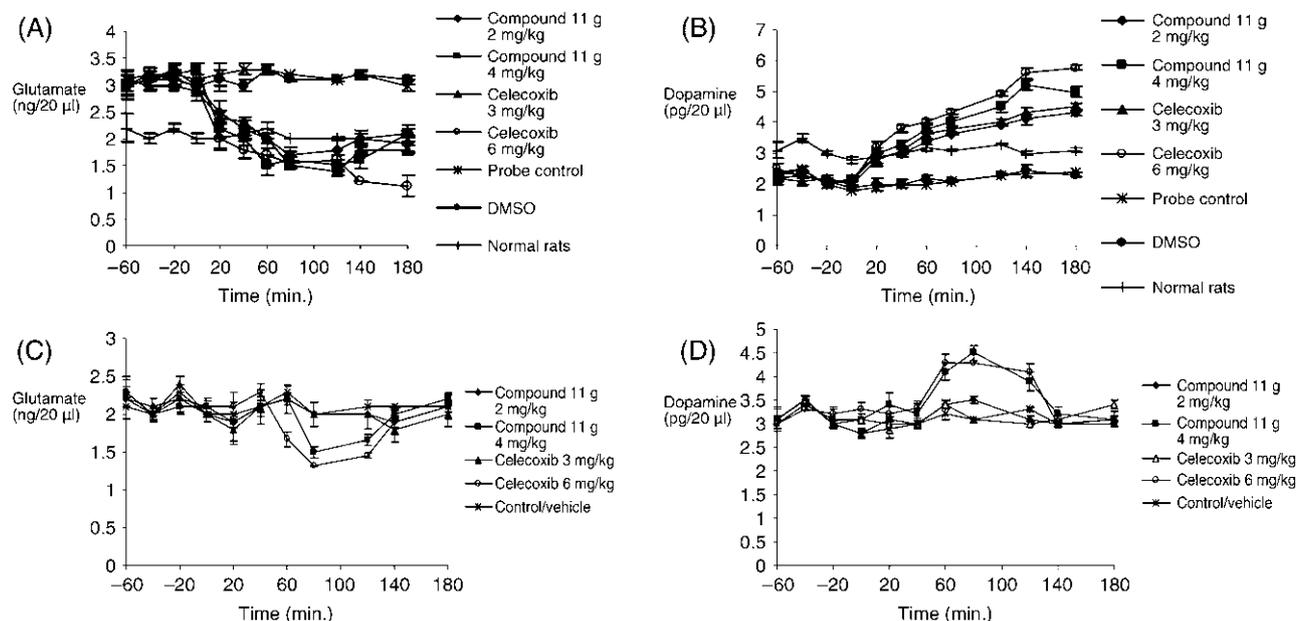


Fig. 1. (A) Effects of selective cyclooxygenase-2 (COX-2) inhibitors on striatal glutamatergic neurotransmission of hemiparkinsonian rats. All doses were observed to be modified significantly the neurotransmission especially during the 20–180 min. after COX-2 inhibitors administration and also at or after 40 min. the glutamate level achieved to the normal and more than it. (B) Effects of selective COX-2 inhibitors on striatal dopaminergic neurotransmission of hemiparkinsonian rats. All doses were observed to be modified significantly the neurotransmission especially during the 20–180 min after COX-2 inhibitors administration and also at or after 20 min. the dopamine level achieved to the normal and more than it. (C) Effects of selective COX-2 inhibitors on striatal glutamatergic neurotransmission of normal rats. Only the high doses of COX-2 inhibitors were seen to be modified significantly the glutamatergic neurotransmission during 80–120 min. (D) Effects of selective COX-2 inhibitors on striatal dopaminergic neurotransmission of normal rats. Only the high doses of COX-2 inhibitors were seen to be modified significantly the dopaminergic neurotransmission during 80–120 min.

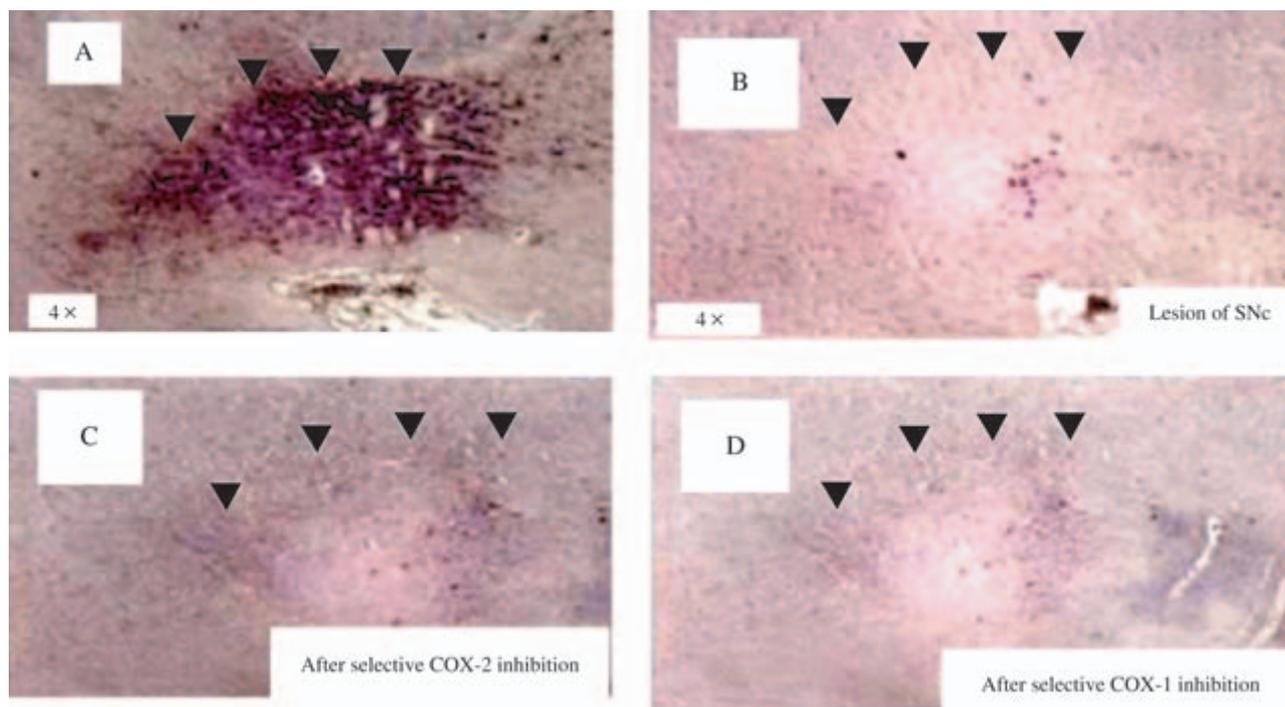


Fig. 2. (A) SNc under light microscope in normal rats; (B) lesioned SNc before any treatment; (C) lesioned SNc after COX-2 inhibition; and (D) lesioned SNc after COX-1 inhibition. All magnification $\times 4$.

mesencephalon also containing glial cells, an experimental preparation that reflected the cellular composition of the brain well, thus useful in the study of neuro-inflammation. Incubation with aspirin, paracetamol or ibuprofen protected dopaminergic neurons against glutamate toxicity. The study considered as indices the reduction of the decrease in dopaminergic uptake caused by glutamate, and the attenuation of the tyrosine hydroxylase-positive cells loss. Among the NSAIDs tested, ibuprofen was the most effective and surprisingly increased the number of dopaminergic cells in basal condition most likely protecting them from the excitotoxicity associated with culture medium change. In the present study, we showed that only high doses of COX-2 inhibitors were able to modify the glutamate-dopamine levels. It has been shown that normally COX-2 is expressed in low levels in nigral dopaminergic neurons, but becomes up-regulated in both patients and experimental Parkinson's disease models [15]. This suggests that different levels of COX-2 in normal and SNc-lesioned rats may explain the aforementioned observations. The changes in other striatal neurotransmitters such as the cholinergic system after COX-2- or COX-1-selective inhibition should be investigated in future experiments in order to clarify the role of COX in other striatal neurotransmissions.

Acknowledgements

Shahid Beheshti, Jondishapour and Tehran Universities of Medical Sciences supported this study. The authors would like to thank Professor Yi Fan, Laboratory of Neuropharmacology, Department of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, China, for his helpful and invaluable guidance.

References

- Minghetti L. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain disease. *J Neuropathol Exp Neurol* 2004;**63**:901–10.
- Shafiee Ardestani M, Fathi Moghaddam H, Hemmati AA. Effects of Aspirin and Celecoxib on rigidity in a rat model of Parkinson's disease. *J Biol Sci* 2007;**21**:3853–8.
- Teismann P, Tieu K, Choi DK, Wu DC, Naini A, Hunot S *et al.* Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proc Natl Acad Sci USA* 2003;**100**:5473–8.
- Tyurina YY, Kapralov AA, Jiang J, Borisenko GG, Potapovich AI, Sorokin A *et al.* Oxidation and cytotoxicity of 6-OHDA are mediated by reactive intermediates of COX-2 overexpressed in PC12 cells. *Brain Res* 2006;**1093**:71–82.
- Chen C, Bazan NG. Lipid signaling: sleep, synaptic plasticity, and neuroprotection. *Prostaglandins Other Lipid Mediat* 2005;**77**:65–76.
- Cole-Edwards KK, Bazan NG. Lipid signaling in experimental epilepsy. *Neurochem Res* 2005;**30**:847–53.
- Allain H, Bentue-Ferrer DL, Akwa Y. Disease-modifying drugs and Parkinson's disease. *Prog Neurobiol* 2008;**84**:25–39.
- Navidpour L, Shadnia H, Shafaroodi H, Amini M, Dehpour AR, Shafiee A. Design, synthesis, and biological evaluation of substituted 2-alkylthio-1,5-diarylimidazoles as selective COX-2 inhibitors. *Bioorg Med Chem* 2007;**15**:1976–82.
- Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego, CA, 1997.
- Duran M, Alfonso B, Arias A. Determination of biogenic amines in rat brain dialysates by high performance liquid chromatography. *J Liq Chromatogr Relat Technol* 1998;**21**:2799–811.
- Hao Y, Yang JY, Guo M, Wu CF, Wu MF. Morphine decreases extracellular levels of glutamate in the anterior cingulate cortex: an *in vivo* microdialysis study in freely moving rats. *Brain Res* 2005;**1040**:191–6.
- Shafiee Ardestani M, Hasan M, Sadeghzadeh N. Effects of dexamethasone and betamethasone as COX-2 gene expression inhibitors on rigidity in a rat model of Parkinson's disease. *Indian J Pharmacol* 2007;**39**:235–9.
- Katzung BG. *Basic and Clinical Pharmacology*. Appleton and Lange, New York, 2004;298–311, 577 and 643–6.
- Riechman W, Hokin LE. Acetylcholine releases prostaglandins from brain slices incubated *in vitro*. *J Neurochem* 1987;**49**:1221–61.
- Casper D, Yaparalvi U, Rempel N, Werner P. Ibuprofen protects dopaminergic neurons against glutamate toxicity *in vitro*. *Neurosci Lett* 2000;**289**:201–4.