

Role of serotonin in cerebral oxidative stress in rats

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Abstract. Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter synthesized by the aromatic amino acid decarboxylase using 5-hydroxytryptophan (5-HTP) as a substrate. It was recently shown that serotonin and its precursor have powerful antioxidant properties. The aim of this study was to evaluate the effect of reduction in 5-HT levels by para-chlorophenylalanine (pCPA) and their restoration by 5-HTP administration on lipid peroxidation and antioxidant status in rat brain. Serotonin levels were decreased by p-chlorophenylalanine administration. The effect of p-chlorophenylalanine was counteracted by the intraperitoneal administration of 5-hydroxytryptophan. We evaluated the concentration of serotonin, malonyl dialdehyde and the status of antioxidants (GSH, catalase and superoxide dismutase) in brain. The results showed that p-chlorophenylalanine (300 mg/kg) induced a depletion of serotonin concentration and antioxidant status, as well as enhancing malonyl dialdehyde concentration in brain. The exogenous administration of 5-hydroxytryptophan prevented all effects induced by p-chlorophenylalanine in brain tissue. The recovery of the neurotransmitter concentration in brain was related to the reduction of lipid peroxide generation and improved antioxidant status. In conclusion, our study supports the view that the antioxidant properties of serotonin protect against basal oxidative stress in brain.

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INTRODUCTION

The serotonergic system regulates mood, emotion, sleep and appetite in mammals. Serotonin or 5-hydroxytryptamine (5-HT) is synthesized by the aromatic aminoacid decarboxylase from 5-hydroxytryptophan (5-HTP). 5-HTP is produced by tryptophan hydroxylase using tryptophan as precursor. The molecule para-chlorophenylalanine (pCPA) inhibits cerebral 5-HT synthesis through the inhibition of tryptophan hydroxylase (Avale et al. 2004, Park et al. 1994). In clinical practice, pCPA has been administered to reduce the unwanted side effect of 5-HT overproduction in carcinoid syndrome (Engelman et al. 1967) and cisplatin-induced emesis in cancer patients (Alfieri and Cubeddu 1995). Nevertheless, pCPA induces relevant noxious side effects, especially those related to hypersensitivity reactions and psychiatric disorder (Bax et al. 1982, Feldman 1982, Ratnavel et al. 1994). In addition, 5-HT depletion induces neuronal cytoskeletal alterations and produces astrogliosis. The recovery of 5-HT levels is associated with a restoration of neuron structure (Ramos et al. 2000). The administration of pCPA also induces acute renal failure characterized by oliguria, decreased glomerular filtration rate, and salt and creatinine retention (Ikonomov et al. 1990). We demonstrated recently that pCPA exacerbates cerebral and renal oxidative stress induced by gentamicin in rats (Muñoz-Castañeda et al. 2005).

Various experimental studies have shown that 5-HT has relevant antioxidant properties. The molecule has demonstrated powerful free radical scavenging properties generated *in vitro* by its chemical system (Andorn and Pappolla 2001, Herraiz and Galisteo 2004, Horakova et al. 2000, Ng et al. 2000). In addition, tryptophan and 5-HTP have also been used to prevent *in vitro* chemical-induced free radical generation (Cadenas et al. 1989). Nevertheless, the implications of the *in vivo* regulation of 5-HT synthesis on lipid peroxidation and antioxidant status in brain have not been fully demonstrated.

The aim of the present study was to evaluate the effect of 5-HT concentration reduction by pCPA and its restoration by 5-HTP administration on lipid peroxidation and antioxidant status in rat brain.

METHODS

Subjects

Two-month-old male Wistar rats (Charles River, Barcelona, Spain) (250–300 g) received laboratory food (Purina®, Barcelona, Spain) and water *ad libitum*. They were subjected to controlled conditions of temperature (about 22°C) and illumination (12 h light – 12 h dark cycle). All animal care and procedures were in accordance with the European Community Council Directive of 24 November 1986 (86/609/ECC) and the R.D. 223/1988, and were approved by the Bioethics Committee of the Institution.

Procedures

To select effective pCPA doses, the animals ($n=18$) were divided into three groups: Control, pCPA (200 mg/kg) and pCPA (300 mg/kg). pCPA (Sigma, St. Louis, USA) was diluted in physiologic saline and injected intraperitoneally for eight days on alternate days. The animals were sacrificed under anaesthesia. Brain was immediately removed, cleaned of blood clots and visible vascular structures, and homogenated in phosphate buffer solution 100 mM, pH 7.4 (4:1) at moderate intensity by five strokes on ice (Ultra-turrax T25, Teflon-Glass, Janke and Kunkel, IKA-Labortechnik, Staufen, Germany). The homogenate was immediately frozen at -40°C. The 5-HT concentration, malonyl dialdehyde, reduced glutathione (GSH), catalase and superoxide dismutase (SOD) activities were evaluated in brain homogenates.

The second part of the study evaluated the capacity of 5-HTP administration to prevent 5-HT and antioxidant status depletion, as well as malonyl dialdehyde exacerbation induced by effective pCPA dose in rat brain. 5-HTP (300 mg/kg) was diluted in physiologic saline and administered three times daily (8:00 A.M., 12:00 P.M. and 9:00 P.M.) for eight days during the treatment of pCPA (300 mg/kg) in rats. The animals ($n=24$) were divided into four groups: Control, 5-HTP, pCPA and pCPA + 5-HTP. The animals were sacrificed under anesthesia. Cerebral extraction and homogenate were done as described in the first part. The concentration of 5-HT, malonyl dialdehyde and antioxidants were evaluated in brain homogenates.

LIPID PEROXIDES ASSAY

A volume of brain homogenate was diluted in 20 mM Tris-HCl buffer pH 7.4 at 1:10 (v:v) for the measurement of lipid peroxides. The procedure described by the supplier was followed (LPO-586, Bioxytec, Portland, USA). The assay is based on the reaction of a chromogenic reagent, N-methyl-2phenylindole, with lipid peroxides (malonyl dialdehyde, MDA and 4-hydroxyalkenals) at 45°C.

REDUCED GLUTATHIONE ASSAY

GSH was measured using a commercial assay GSH-400 (Bioxytech S.A.). The brain homogenate was diluted in 200 mM potassium phosphate pH 7.4 at 1:10 (v/v) for the measurement of GSH. Following the instructions of the supplier, all mercaptans present in the sample react with 4-chloro-1-methyl-7-trifluoromethyl-quinolinium methylsulfate in a first chemical reaction. The second step consisted of the β -elimination reaction under alkaline conditions of the product from the first reaction into a chromogenic thione-derived product.

CATALASE AND SUPEROXIDE DISMUTASE ASSAY

The brain homogenate was diluted in 50 mM phosphate buffer pH 7.4 at 1:10 (v/v) for the measurement of catalase and SOD. Catalase was determined following the method described by Aebi (1984). The procedure is based on the measurement of the decomposition rate of H₂O₂ (10 mM) to water and molecular oxygen measured at 240 nm. SOD activity was assessed by the method of Sun and coauthors (1988). The assay is based on the inhibition of nitroblue tetrazolium reduction upon superoxide generation by xanthine-xanthine oxidase system. The activity of SOD was assessed at 420 nm. Activities of enzymes were expressed as AU/g of tissue (AU= Activity Units).

SEROTONIN EXPRESSION

5-HT content was measured following the method described by Snyder and coauthors (1965). Briefly, 5-HT was extracted with 1-butanol in a salt saturated solution (pH 10) and further returned to an aqueous solution (pH 7.0) by the addition of heptane. 5-HT reacted with

ninhydrin to yield a fluorescent product using excitation wavelength 385 nm and emission wavelength 490 nm. Fluorescence was measured using a Zuzi spectrofluorometer model 930 (Zuzi, Beijing, China) and its values expressed as percentage of control values.

STATISTICAL ANALYSIS

Results were expressed as percentage or means \pm SEM. Data followed a normal distribution using the Shapiro-Wilks test. Multiple comparisons were carried out using one-way analysis of variance (ANOVA) followed by the Bonferroni test. Significance was set at $P \leq 0.05$.

RESULTS

Effects of pCPA on serotonin content and oxidative stress in brain

The administration of pCPA induced a dose-dependent reduction of 5-HT content in brain (Table I). In consequence, the administration of pCPA (300 mg/kg) induced greater inhibition of 5-HT content in brain than pCPA (200 mg/kg) administration (86% vs. 82%, respectively).

The results showed that pCPA induced a dose-dependent response in malonyl dialdehyde levels (56%

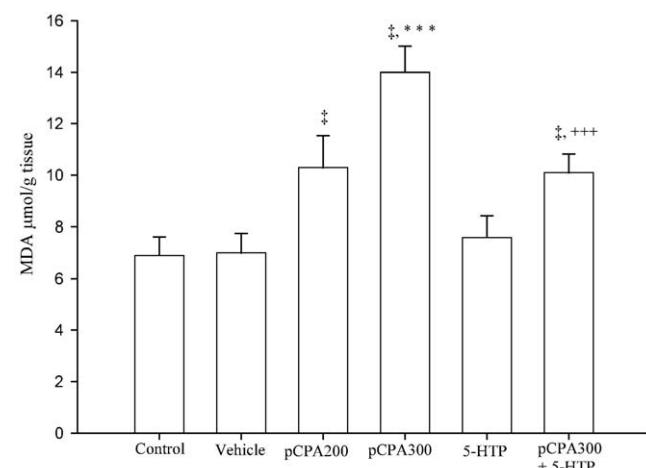


Fig. 1. Effect of various doses of para-chlorophenylalanine (pCPA) and 5-hydroxy-tryptophan (5-HTP) administration on the concentration of lipid peroxides in brain tissue. Bars represent mean \pm SEM. (‡) $P \leq 0.001$ vs. Control group; (*** $P \leq 0.001$ vs. pCPA200; (++) $P \leq 0.001$ vs. pCPA300.

Table I

Regulation of 5-HT, reduced glutathione (GSH), catalase and SOD content in brain of rats treated with various doses of para-chlorophenylalanine (pCPA)

	5-HT% ng 5-HT/g tissue	GSH mmol/g tissue	CATAU/g tissue	SODAU/g tissue
Control	100	0.59 ± 0.080	0.072 ± 0.0026	2.2 ± 0.18
Vehicle	99	0.61 ± 0.063	0.069 ± 0.0094	2.2 ± 0.23
pCPA [200]	18	0.25 ± 0.031‡	0.065 ± 0.0026‡	2.0 ± 0.13
pCPA [300]	14	0.19 ± 0.042‡, *	0.035 ± 0.0042‡, ***	1.3 ± 0.05‡, ***

Values are expressed as percentage (5-HT) and mean ± SEM (GSH, CAT, SOD), $n=6$ rats per group. (‡) $P \leq 0.001$ vs. Control; (*** $P \leq 0.001$ and (*) $P \leq 0.05$ vs. pCPA 200.

and 102% in pCPA200 and pCPA300, respectively) and reduced glutathione level (42% and 32% in pCPA200 and pCPA300, respectively), SOD and CAT activities in brain (Fig. 1 and Table I). pCPA administration had a dose-dependent effect on SOD and CAT activities. In this respect, pCPA enhanced malonyl dialdehyde concentration, and depleted GSH content, as well as SOD and CAT activities in brain homogenates, in comparison with control group ($P \leq 0.001$). In addition, the administration of the higher dose of pCPA (300 mg/kg) induced more powerful oxidative stress in brain (alterations in MDA, GSH) than the lower dose in pCPA (200 mg/kg)-treated rats ($P \leq 0.001$).

EFFECT OF 5-HYDROXYTRYPTOPHAN ADMINISTRATION ON 5-HT CONTENT AND OXIDATIVE STRESS IN BRAIN FROM PARA-CHLOROPHENYLALANINE-TREATED RATS

5-HTP was administered to rats submitted to the highest concentration of 5-HT synthesis inhibitor (300 mg/kg). 5-HTP enhanced significantly 5-HT concentration in brain in control and pCPA-treated rats (Table II). The administration of 5-HTP reduced malonyl dialdehyde concentration (56%) (Fig. 1) and increased GSH content (81%) (Table II) in brain of pCPA-treated rats ($P \leq 0.001$).

DISCUSSION

The administration of pCPA induced unwanted side effects related to toxicity in the nervous system in experimental animals (Bax et al. 1982, Feldman 1982, Ratnavel et al. 1994). Our results show that oxidative

stress induced by pCPA is sensitive to the pCPA dose. Thus, the administration of 300 mg/kg weight of pCPA causes significantly greater cerebral oxidative stress than the 200 mg/kg dose does. The percentage of 5-HT inhibition is also dependent on the pCPA concentration (pCPA200 82% and pCPA300 86%). The increase observed in oxidative stress may be due to pCPA being a neurotoxic molecule that triggers cerebral oxidative stress (Tables I and II, Fig. 1). In this sense, small changes in 5-HT concentration diminish significantly the intensity of oxidative stress. This can be explained by the antioxidant properties of 5-HT against cerebral oxidative stress. We think that this oxidative stress may be related to the absence of an important antioxidant such as 5-HT or melatonin, as well as to the cerebral

Table II

Effect of 5-hydroxytryptophan (5-HTP) administration on the reduction of 5-HT and reduced glutathione (GSH) content induced by para-chlorophenylalanine (pCPA) in brain tissue

	5-HT % ng 5-HT/g tissue	GSH mmol/g tissue
Control	100	0.63 ± 0.048
Vehicle	99	0.60 ± 0.007
5-HTP	129	0.60 ± 0.030
pCPA [300]	14	0.21 ± 0.042‡
pCPA [300]+ 5-HTP	57	0.48 ± 0.022‡, +++

Values are expressed as percentage (5-HT) and mean ± SEM (GSH). (‡) $P \leq 0.001$ vs. Control; (++) $P \leq 0.001$ vs. pCPA 200.

injury induced by pCPA. In this sense, 5-HT and its precursor (5-HTP) have demonstrated powerful scavenging properties against free radicals generated using the chemical system *in vitro* (Andorn and Pappolla 2001, Betten et al. 2001, Cadenas et al. 1989, Herraiz and Galisteo 2004, Horakova et al. 2000, Liu and Mori 1993, Ng et al. 2000, Wang et al. 2001). The presence of hydroxylated phenolic rings in the structure of these molecules may be responsible for their antioxidant capacity (Hadi et al. 2002, Rice-Evans et al. 1997). However, a 5-HT-receptor cell mediated mechanism has also been suggested (Ahlemeyer and Kriegstein 1977, Ahlemeyer et al. 2000, Migheli et al. 1999). This alternative cannot be ruled out on the basis of data obtained in the present study.

Our study shows that the *in vivo* depletion of 5-HT induces susceptibility of brain to oxidative stress in rats (Fig. 1, Tables I and II). In addition, the administration of 5-HTP reverts the effects produced by pCPA, decreasing MDA and increasing CAT, SOD and GSH (Table II and Fig 1).

A further demonstration of the antioxidant properties of 5-HT is related to the involvement of oxidative stress in neurodegenerative disorders. It is generally accepted that enhanced susceptibility to oxidative stress is relevant to chronic neurodegenerative disorders during aging (Gotz et al. 1994). Arivazhagan and Panneerselvam (2002) have shown that 5-HT levels decrease in rat brains during aging. The treatment of aged rats with the antioxidant α -lipoic acid increases 5-HT levels, suggesting that 5-HT depletion during aging might be due to oxidative stress. Mattson et al. (2004) observed that 5-HT cooperates with brain-derived neurotrophic factor (BDNF) and protects against aging, Alzheimer's disease and Huntington's disease.

However, in some pathological conditions, a noxious effect of 5-HT overproduction in brain has been observed. Catecholamine neurotransmitters and tryptophan derivates, including 5-HT, show free radical scavenging and neuroprotective properties. However, they are also readily oxidized to toxic products catalyzed by peroxidase, prostaglandin H synthase, xanthine oxidase, tyrosinase or cytochrome P450, which have been found in neural tissues (Galzigna et al. 1999). Catecholamine-oxidized metabolites, such as 5,5'-dihydroxy-4,4'-bitryptamine, have been implicated in neurodegenerative diseases. The induction of high catecholamine production is related to the accumulation of toxic oxidized metabolites that might contribute to

neurochemical and neurobiochemical changes associated with neurological diseases (Wrona et al. 1992). Nevertheless, Schmued and coauthors (1999) suggested that a rise in 5-HT levels is not in itself sufficient for neurotoxicity, and additional factors such as hyperthermia, regional specificity of 5-HT receptor subtypes, blood flow and/or neuronal networks may be involved. To clarify the role of 5-HT against oxidative stress and neurological disorder, further preliminary studies using the appropriate experimental models are required.

CONCLUSION

This study shows that the reduction of 5-HT content by pCPA administration induced dose-dependent lipid peroxidation and antioxidant status depletion in brain. In consequence, in clinical situations accompanied by a reduction of 5-HT synthesis, brain tissue is especially susceptible to the induction of oxidative-dependent tissue damage.

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