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Cocaine alters catalase activity in prefrontal cortex and striatum of mice

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Abstract

Catalase is one of the enzymes that convert hydrogen peroxide (H_2O_2) to H_2O presenting a protective role against free radicals. In this study, catalase activity was determined in homogenates of striatum (ST) and prefrontal cortex (PFC) in order to examine the participation of oxidative stress (OS) on cocaine actions in mice brain. Male Swiss mice were injected (i.p.) with cocaine at low (10 and 30 mg/kg) and high doses (90 mg/kg), and observed for 1 h. After cocaine overdose (90 mg/kg) some animals presented only status epilepticus (SE) while others died after seizures. These animals were dissected and divided in two groups, SE and death. Catalase activity was also determined after pretreatment with the anticonvulsant drug, diazepam, alone or injected before cocaine 90 mg/kg, and after seizures induced by a high dose of bupropion, a known inhibitor of NE and DA reuptake used for comparison. Results showed a decrease in catalase activity of the PFC and ST after SE and death induced by cocaine and bupropion overdoses. Cocaine at low doses decreased the enzyme activity only in ST. Diazepam treatment alone and before cocaine overdose did not interfere with catalase activity. This reduction in catalase activity may reflect an increase in H_2O_2 content in PFC and ST. Previous data reports that H_2O_2 inhibits dopamine transporter activity, suggesting that the decrease in catalase activity may potentiate the toxic mechanism of drugs that inhibit monoamines reuptake. As far as we know, this is the first report showing an involvement of OS in the cocaine's central mechanism of action.

Keywords: Cocaine-induced seizures; Status epilepticus; Cocaine-induced lethality; Catalase; Bupropion; Prefrontal cortex; Striatum

Cocaine overdose is related to toxic effects, such as convulsions and death. Cocaine-induced seizures and lethality seems to be mediated by distinct neurotransmitter systems [21,22]. It is known that cocaine-induced seizures are enhanced by drugs that stimulates D_1 receptors and inhibited by D_1 antagonists, as well as by D_2 agonists [27]. Recent data from our laboratory also showed that monoamine levels, such as dopamine are altered after cocaine-induced seizures and lethality in prefrontal cortex and striatum [18]. Serotonin 5-HT_2 receptor antagonists, like cinanserin and ketanserin [21] were found to decrease cocaine-induced seizures, while other data indicate that inhibition mediated by γ-aminobutyric acid is a major target for the central action of cocaine, resulting in seizures [29]. Cocaine-induced lethality as referred previously [22] seems to involve concurrent interactions of this drug with dopaminergic, muscarinic and sigma opioid neurotransmitter systems.

Although several neurotransmitter systems may participate in cocaine-induced seizures and lethality there is a lack of information in the literature about the participation of free radicals in this brain process. Currently, free radicals are involved in the pathogenesis of various diseases including atherosclerosis, diabetes mellitus, stroke, inflammatory

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diseases and cancer [20,19,12,10,26] as well as in generalized epilepsy [1]. The excessive production of free radicals is called oxidative stress (OS). The OS in the brain is facilitated, more than in other tissues, because it contains large quantities of oxidizable lipids and metals, and has comparatively less antioxidant mechanisms.

Catalase is one of the enzymes that convert hydrogen peroxide (H_2O_2) to H_2O. The H_2O_2 is not a free radical itself, but it can be rapidly decomposed via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$) or can be interconverted via the so-called Haber–Weiss reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$), to form hydroxyl radicals [7].

It is also known that cocaine increases OS and this can lead to a neurodegeneration [9,24]. Brain studies with multiple low doses of cocaine generated reactive oxygen species (ROS) such as hydroxyl radicals which are likely formed by the drug metabolites [16].

Based on the facts described above, the present study was performed to evaluate the participation of catalase in cocaine mechanism of action, as well as in cocaine-induced seizures and lethality.

Experimentally naive, male Swiss mice weighing 20–30 g housed in home cages in a light/dark cycle and with water and food ad libitum were used. Experiments were conducted between 1:00 and 5:00 p.m. and performed according to the Guide for Care and Use of Laboratory Animals provided by the NIH, USA. Experimental groups consisted of 6–13 animals per each drug and dose and all mice were used only once.

The following drugs were used: cocaine, obtained from Federal Police of Ceará, diazepam (Dienpax®, Sanofi-Synthelabo, Brazil), bupropion (Zyban®, Glaxo-Wellcome, Grenford, Middlesex, UK). Mice were injected with cocaine at low (10 and 30 mg/kg, i.p.; COC 10 and COC 30, respectively) doses and were decapitated 1 h after treatment. Injection of cocaine at a high dose (90 mg/kg, i.p.; COC 90) induced seizures within 2–4 min after administration. After the onset of seizures 60% of the animals started a status epilepticus that lasted approximately 30 min. These animals were observed for 1 h after cocaine injection and then were decapitated and separated in a group called status epilepticus (SE). The other 40% seized and died 5–15 min after cocaine administration and were immediately decapitated and separated in a group called death.

To assess the effect of pretreatment with anticonvulsants before COC 90 on the catalase activity, animals were treated with diazepam 10 mg/kg (DZP 10) and 30 min later received cocaine 90 mg/kg. Another group of animals received only DZP 10. Mice were sacrificed 1 h after cocaine administration.

The catalase activity was also determined after seizures induced by a high dose of bupropion (150 mg/kg, i.p.; BUP) that similarly to cocaine is also an inhibitor of DA and NE reuptakes. The animals were dissected 1 h after bupropion administration. Controls for all experiments received saline 0.9% and were killed 1 h after injection. After interval times all animals were decapitated, their brains were removed and striatum and prefrontal cortex were dissected for the determination of catalase activity.

The catalase activity was measured by the method that employs hydrogen peroxide to generate H_2O and O_2 [3]. The activity was measured by the degree of this reaction. The assay mixture contained 0.3 ml of hydrogen peroxide in 50 ml of 0.05 M phosphate buffer, pH 7.0. The sample aliquot (20 μl) was added to 980 μl of the substrate mixture. Initial and final absorbances were recorded at 230 nm after 1 and 6 min, respectively. A standard curve was established using purified catalase (Sigma, MO, USA) under identical conditions. All samples were diluted with 0.1 mmol/l phosphate buffer (pH 7.0) to provoke a 50% inhibition of the diluent rate (i.e. the uninhibited reaction). Protein was determined using bovine serum albumin as standard [17]. Results (mM/min/mg protein) are expressed as mean ± S.E.M. of 6–13 animals/group performed in duplicate. Statistical analyses were estimated using unpaired "*t*-test" (for comparisons between two groups) and ANOVA followed by Student–Newman Keuls test (for comparisons among more than two groups). The index of probability of 0.05 ($p < 0.05$) or less was considered significant in comparative analysis.

All results were compared to control animals. The catalase activity in the striatum as shown in Fig. 1A decreased around 60% after COC 10 ($p < 0.001$) and 30 ($p < 0.001$)

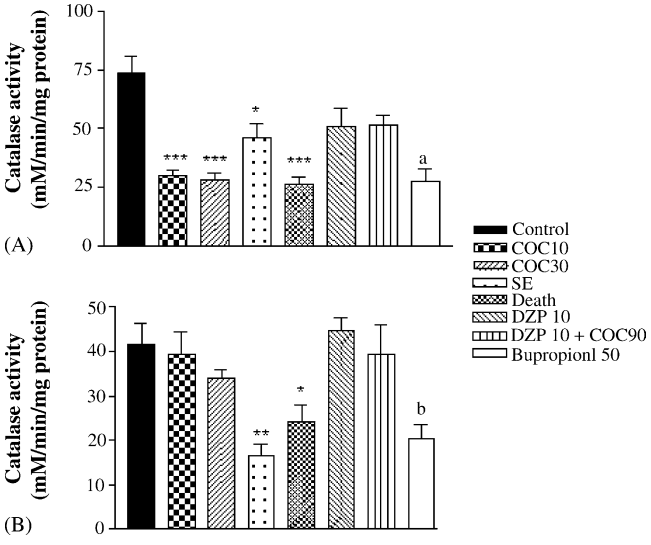

Fig. 1. Catalase activity in striatum (A) and prefrontal cortex (B) of mice. Separate groups of animals ($n = 6$–13) were treated with saline (controls), cocaine in low (COC 10 and 30 mg/kg, i.p.) and high doses (COC 90 mg/kg, i.p. – separated as status epilepticus (SE) and death), pretreated with the anticonvulsant drug, diazepam 10 mg/kg, i.p. (DZP 10) alone or 30 min before cocaine 90 mg/kg administration, and submitted to bupropion overdose (150 mg/kg). One hour after treatment the animals had striatum and prefrontal cortex dissected for catalase activity determination. Each bar represents mean ± S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to control animals (ANOVA followed by Student–Newman Keuls test); $^{a}p = 0.0005$, $^{b}p = 0.01$ as compared to control animals ("*t*-test").