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LC/MS/MS evaluation of cocaine and its metabolites in different brain areas, peripheral organs and plasma in cocaine self-administering rats

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Abstract:
Background: We employed a cocaine intravenous self-administration model based on positive reinforcement of animals' instrumental reactions (i.e., lever pressing) rewarded by a dose of the drug. We also carried out simultaneous characterization of the pharmacokinetics of cocaine and its metabolites in rats during withdrawal; in this part of the experiments, we investigated the cocaine (2 mg/kg, *iv*)-induced changes in the distribution, rate constant, clearance and $t_{1/2}$ of the parent drug and its metabolites in different structures of the brain and in peripheral tissues.
Methods: By using liquid chromatography-tandem mass spectrometry (LC/MS/MS) we measured the levels of cocaine and its major metabolites.
Results: Our results demonstrate differences in the levels of cocaine after cocaine self-administration in the rat, with the highest concentration seen in the striatum and the lowest in the cerebellum. Cocaine metabolites determined in the rat brain remained at very low levels (benzoylecgonine), irrespectively of the brain area, whereas the norcocaine concentration varied from 1.56 μg/g (the nucleus accumbens) to 2.73 μg/g (the striatum).
Conclusion: A tandem LC/MS/MS is a valid method for evaluation of brain and peripheral levels of cocaine and its metabolites. Our results demonstrate brain area-dependent differences in the levels of cocaine after its self-administration in the rat. There were also differences in pharmacokinetic parameters among the brain areas and peripheral tissues following a bolus *iv* injection of cocaine to rats withdrawn from cocaine; among brain structures the slowest metabolic rate was detected for the striatum.

Key words:
cocaine, norcocaine, benzoylecgonine, metabolism, brain structures, LC/MS/MS, kinetic

Abbreviations: BE – benzoylecgonine, CER – cerebellum, EME – ecgonine methyl ester, FC – frontal cortex, HIP – hippocampus, LC/MS/MS – liquid chromatography with tandem mass spectrometry, NAC – nucleus accumbens, NC – norcocaine, PFC – prefrontal cortex, STR – striatum

Introduction

Cocaine is an abused psychostimulant and cocaine addiction is a grave socio-medical burden affecting the

whole human population (UNDOC, 2010). Being a lipophilic compound, cocaine easily penetrates the blood-brain barrier and both in the periphery and in the brain its mechanism of action includes interaction with aminergic (dopamine, noradrenaline and serotonin) neurotransmitter systems *via* binding to their transporter sites and reuptake inhibition [3, 11, 33, 38]. Apart from its direct pharmacological actions, cocaine is degraded in the blood, peripheral tissues and brain to many pharmacologically active metabolites (Fig. 1). Studies in humans and animals have demonstrated two major metabolic transformations of cocaine: (i) hydrolysis by esterases in blood and tissues (accounting for 80–90% of total elimination) and (ii) oxidation by microsomal mixed-function oxidases (i.e., cytochrome P-450 (CYP) enzymes), mainly in the liver [26, 43]. The hydrolytic pathway of cocaine metabolism in the body includes rapid inactivation of the ester bonds to form benzoylecgonine (BE) and ecgonine methyl ester (EME) (Fig. 1). BE is formed by nonenzymatic hydrolysis, while EME formation is catalyzed by esterases localized in the liver and serum [42]. Both BE and EME are bioactive metabolites of cocaine [30, 41], less toxic than the parent drug [46] and having an approximately five times longer half-life ($t_{1/2}$) in biological matrices than cocaine [7, 29, 45, 46]. Ten percent of cocaine administered to the body is catalyzed by the liver microsomal reactions *via* CYP2B1 enzymes in rats [2], and by CYP3A enzymes in mice and humans [34–36] leading to the formation of a pharmacologically active *N*-demethylated metabolite, norcocaine (NC). NC has a greater tissue affinity than cocaine [1, 2, 46] but the pharmacologic or toxic effects of cocaine and norcocaine are similar [32, 48]. Like cocaine, lipophilic NC can penetrate to the brain and has been isolated from brain tissue minutes after systemic administration [24, 31] indicating that this metabolite is either formed in or enters the brain.

The aim of the present study was to measure the levels of cocaine and its major metabolites (BE and NC) in several brain structures (dorsal striatum (STR), nucleus accumbens (NAC), prefrontal cortex (PFC), frontal cortex (FC), hippocampus (HIP) and cerebellum (CER)), some peripheral tissues (heart, liver and kidney) and in serum in rats addicted to cocaine. To this end, we employed a cocaine intravenous self-administration model based on positive reinforcement of animals' instrumental reactions (i.e., lever pressing) rewarded by a dose of the drug. We also carried out simultaneous characterization of the pharmacokinetics of cocaine and NC in rats with stabilized cocaine self-administration and extinction; in this part of experiments we investigated the cocaine (2 mg/kg, *iv*)-induced changes in the distribution, rate constant, clearance and $t_{1/2}$ of the parent drug and its metabolites in different structures of brain and peripheral tissues. The cocaine, BE and NC levels were measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS).

Materials and Methods

Animals

Male Wistar rats (280–300 g; n = 26) delivered by a licensed breeder (Charles River, Germany) were housed individually in standard plastic rodent cages in a colony room maintained at 20 ± 1°C and at 40–50% humidity under a 12-h light-dark cycle (lights on at 6:00). Animals had free access to standard animal food and water during the 7-day habituation period. All experiments were conducted during the light phase of the light-dark cycle (between 8:00–15:00) and were carried out in accordance with the National Institutes of *Health Guide for the Care and Use of Laboratory Animals* and with approval of the Bioethics Commission as compliant with the Polish Law (21 August 1997). The animals were experimentally naive.

Drugs

Cocaine hydrochloride (Sigma-Aldrich, USA), dissolved in sterile 0.9% NaCl, was used. Cocaine was given *iv* (0.1 ml/infusion or 1 ml/kg).

Cocaine self-administration procedure

Rats (n = 10) were trained to press the lever of standard operant conditioning chambers (Med-Associates, USA) under a fixed ratio 5 schedule of water reinforcement. Two days following "lever-press" training and free access to water, the rats were chronically implanted with a silastic catheter in the external right jugular vein, as described previously [17]. Catheters were flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml) and 0.1 ml of solution of cephazolin (10 mg/ml; Biochemie GmbH, Austria). After a 10-day recovery period, all animals were wa-

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