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Changes in endocannabinoid and N-acylethanolamine levels in rat brain structures following cocaine self-administration and extinction training

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ABSTRACT

Preclinical investigations have demonstrated that drugs of abuse alter the levels of lipid-based signalling molecules, including endocannabinoids (eCBs) and N-acylethanolamines (NAEs), in the rodent brain. In addition, several drugs targeting eCBs and/or NAEs are implicated in reward and/or seeking behaviours related to the stimulation of dopamine systems in the brain.

In our study, the brain levels of eCBs (anandamide (AEA) and 2-arachidonoylglycerol (2-AG)) and NAEs (oleoylethanolamide (OEA) and palmitoylethanolamide (PEA)) were analyzed via an LC-MS/MS method in selected brain structures of rats during cocaine self-administration and after extinction training according to the "yoked" control procedure.

Repeated (14 days) cocaine (0.5 mg/kg/infusion) self-administration and yoked drug delivery resulted in a significant decrease (ca. 52%) in AEA levels in the cerebellum, whereas levels of 2-AG increased in the frontal cortex, the hippocampus and the cerebellum and decreased in the hippocampus and the dorsal striatum. In addition, we detected increases (>150%) in the levels of OEA and PEA in the limbic areas in both cocaine treated groups, as well as an increase in the tissue levels of OEA in the dorsal striatum in only the yoked cocaine group and increases in the tissue levels of PEA in the dorsal striatum (both cocaine groups) and the nucleus accumbens (yoked cocaine group only). Compared to the yoked saline control group, extinction training (10 days) resulted in a potent reduction in AEA levels in the frontal cortex, the hippocampus and the nucleus accumbens and in 2-AG levels in the hippocampus, the dorsal striatum and the cerebellum. The decreases in the limbic and subcortical areas were more apparent for rats that self-administered cocaine. Following extinction, there was a region-specific change in the levels of NAEs in rats previously injected with cocaine; a potent increase (ca. 100%) in the levels of OEA and PEA was detected in the prefrontal cortex and the hippocampus, whilst a drop was noted in the striatal areas versus yoked saline yoked animals.

Our findings support the previous pharmacological evidence that the eCB system and NAEs are involved in reinforcement and extinction of positively reinforced behaviours and that these lipid-derived molecules may represent promising targets for the development of new treatments for drug addiction.

1. Introduction

Drug addiction is an extremely serious problem, both in terms of the proper functioning of the body and in terms of specific social behaviour that is often in conflict with the law. Amongst addictive drugs, cocaine belongs to the group of psychostimulants with a high abuse potential due to its induction of euphoric feelings, friendliness, empathy and hyperactivity (Kreek et al., 2012). The mechanism of action of cocaine includes interaction with monoaminergic (dopamine, noradrenalin and serotonin) neurotransmitter systems via inhibition of monoamine reuptake (Bossert et al., 2005; Nestler, 2004). The acute reinforcing effects of cocaine are associated with a marked increase in dopaminergic neurotransmission and an indirect activation of both D_1 and D_2 receptors at the synaptic termini of mesolimbic dopaminergic neurons (Di Chiara, 1995; Thomsen et al., 2009). Recent results indicate that cocaine – in addition to monoamine transporter binding – induces direct and/or indirect allosteric stimulation of D_2 receptors. In fact, cocaine enhanced the ability of the D_2-like receptor agonist quinpirole to reduce K^+-evoked [^{3}H]DA efflux from rat striatal synaptosomes (Ferraro et al., 2010). Cocaine also increased membrane-associated D_2 receptor immunoreactivity in CHO cell lines lacking the dopamine transporter (Genedani et al., 2010) and the efficacy of dopamine to stimulate the binding of GTPγS to striatal D_2-like receptors (Ferraro et al., 2012). In vivo studies also revealed that low concentrations of cocaine

Abbreviations: AEA, anandamide; 2-AG, 2-arachidonoylglycerol; eCBs, endocannabinoids; LC-MS/MS, liquid chromatography tandem mass spectrometry; NAEs, N-acylethanolamines; OEA, oleoylethanolamide; PEA, palmitoylethanolamide.

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amplified the quinpirole-induced reduction in accumbal extracellular glutamate levels and hyperlocomotion (Ferraro et al., 2012).

In the late 20th century, a novel inhibitory feedback mechanism counteracting the dopamine-induced facilitation of behavioural and neurochemical functions was discovered. For instance, an in vivo microdialysis study indicated that the striatal administration of quinpirole evoked a marked local increase in the level of anandamide (AEA, N-arachidonoylethanolamine), a lipid-based molecule (Giuffrida et al., 1999), whilst an in vitro study revealed that cocaine or quinpirole perfusion into striatal slices evoked an increase in the AEA level (Centonze et al., 2004). These effects of cocaine were blocked by a D_2 receptor antagonist both in vivo and in vitro. This evidence indicates that endocannabinoids (eCBs) are the downstream effectors of the action of cocaine in the dorsal striatum. These studies also suggest functional interactions between eCBs and dopaminergic systems during striatal signalling. Further preclinical reports have confirmed that the eCB system may play a role in cocaine addiction (Arnold, 2005), especially in the reinstatement of drug-seeking behaviours (Adamczyk et al., 2009; Budzyńska et al., 2009; Shoaib, 2008; Xi et al., 2006).

Aside from AEA, the eCB system includes other endogenous lipid molecules, such as 2-arachidonoylglycerol (2-AG) (Onaivi et al., 2002). Via cleavage from the plasma membrane, synthesis of lipid precursors, including both AEA and 2-AG, is specifically regulated by neuronal activity. Once generated, eCBs primarily act via two receptors, CB_1 and CB_2, to regulate synaptic communication, membrane depolarisation and neurotransmitter release (Di Marzo, 2011; Onaivi et al., 2002; Piomelli, 2003).

It has also been discovered that non-cannabinoid fatty acid ethanolamides may participate in the control of reward-related behaviours (Fu et al., 2008; Hansen and Diep, 2009; Melis et al., 2008). These compounds include oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), which act indirectly on CB receptors and may influence eCB function by competing for catabolic enzymes (Giuffrida et al., 2000). Both ethanolamides also possess neuromodulatory properties as endogenous ligands of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α) and the capsaicin receptor transient receptor potential cation channel subfamily V member 1 (TRPV1) (TRPV1) (Melis et al., 2008).

Investigations of cannabinoid and non-cannabinoid fatty acid ethanolamides (also known as N-acylethanolamines or acylethanolamides; NAEs) in rat brain structures following repeated cocaine administration in in vivo models are very limited, and the evaluation of active cocaine exposure via self-administration has not yet been studied. We sought to identify the magnitude of AEA, 2-AG, OEA and PEA levels in several rat brain structures via liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis during maintenance of cocaine self-administration and after drug withdrawal using a yoked-triad procedure. The latter procedure (in which each animal was paired with two rats that served as "yoked" controls, of which one received an injection of saline each time the paired rat self-administered a response-contingent injection of cocaine, and the second received an injection of cocaine in the same manner) allowed us to distinguish between the pharmacological and motivational effects of psychostimulant intake.

2. Materials and methods

2.1. Animals

A group of 48 male Wistar rats (280–300 g) 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 06:00). The animals had free access to standard animal food and water during the 7-day habituation period. Then, the animals were divided into three groups (active, yoked and control, see Table 1) and the rats used in the cocaine self-administration procedures were maintained on limited water during initial training sessions (see below). All of the experiments were conducted during the light phase of the light–dark cycle (between 08:00 and 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 Polish Law (21 August 1997). The animals were experimentally naive.

Table 1
Experimental protocol for behavioural studies.

Group	Maintenance of self-administration	Extinction
	14 days	10 days
1a	Yoked saline	
1b	Yoked cocaine	
1c	Cocaine self-administration	
2a	Yoked saline	Saline
2b	Yoked cocaine	Saline
2c	Cocaine self-administration	Saline

Rats (groups 1c and 2c) were trained to self-administered cocaine (0.5 mg/kg/infusion) during 2-h daily sessions. Groups 1a, 1b and 1c were sacrificed immediately following the last self-administration session, whilst groups 2a, 2b and 2c underwent 10-day extinction and were sacrificed immediately following the last 2-h extinction session.

2.2. Drugs

Cocaine hydrochloride (Sigma-Aldrich, St. Louis, USA), dissolved in sterile 0.9% NaCl and given *iv* (0.1 ml/infusion).

2.3. Behavioural procedures

2.3.1. Cocaine self-administration and extinction training

Rats 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 (Frankowska et al., 2010). The catheters were flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml, Biochemie GmbH, Austria) and 0.1 ml of solution of cephazolin (10 mg/ml; Biochemie GmbH, Austria). There was no problem with catheter patency.

After a 10-day recovery period, all of the animals were water deprived for 18 h and trained to lever press to a fixed ratio 5 schedule of water reinforcement over a 2-h session. The subjects were then given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance) and from that time they were given *ad libitum* water. The house light was illuminated throughout each session. Each completion of five presses on the active lever complex (the fixed ratio 5 schedule) resulted in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) and a 5-s presentation of a stimulus complex (activation of the white stimulus light directly above the active lever and the tone generator, 2000 Hz; 15 dB above ambient noise levels). Following each injection, there was a 20-s time-out period during which responding was recorded but had no programmed consequences. Response on the inactive lever never resulted in cocaine delivery. Acquisition of the conditioned operant response lasted a minimum of 10 days until the subjects met the following criteria: minimum requirement of 22 reinforcements with an average of 6 days, and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of <10% of the average; this selected criterion was based on our prior experiments (Filip, 2005). After the last (2 h) self-administration session, the animals were decapitated.

After 14 days of self-administration (once the rats met the maintenance criterion), a separate group of rats (n = 24) underwent 10-day extinction trials. During extinction, the animals experienced 2-h daily