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Research report

Adrenalectomy prevents behavioural sensitisation of mice to cocaine in a genotype-dependent manner

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Abstract

The objective of the present study was to investigate the contribution of adrenal stress hormones to strain differences in cocaine sensitivity. For this purpose, we have studied sensitisation to the locomotor stimulant effect of cocaine and, in parallel, cocaine-induced corticosterone secretion in two inbred mouse strains: C57BL/6 and DBA/2. Adrenalectomy ('ADX': surgical removal of the adrenal glands) was performed in a subset of animals to investigate the contribution of the adrenals. ADX and SHAM operated mice were subjected to repeated injections of cocaine (15.0 mg/kg) or saline for nine consecutive days, followed by a 5-day withdrawal interval and a saline challenge on day 14. All animals were challenged with 7.5 mg/kg cocaine on day 15.

We report that repeated cocaine exposure induced locomotor sensitisation in both strains, while endocrine sensitisation was only observed in the DBA/2 strain. By contrast, cocaine attenuated corticosterone responses in C57BL/6 mice throughout the sensitisation paradigm. We have therefore identified one strain, the DBA/2 strain, that displays parallel sensitisation of cocaine-induced locomotion and -corticosterone secretion. Most interestingly, ADX prevented locomotor sensitisation only in DBA/2 mice, suggesting that behavioural sensitisation depends on the integrity of adrenal function and on secretion of adrenal glucocorticoids in this strain.

The present results demonstrate that adrenal stress hormones facilitate behavioural sensitisation to cocaine in a genotype-dependent manner and suggest that glucocorticoids contribute to strain differences in psychostimulant sensitivity.

Keywords: Glucocorticoids; Dopamine; Cocaine; Genotype; Locomotor activity; Sensitisation; Adrenalectomy

1. Introduction

Behavioural responses to psychostimulant drugs are characterised by a large degree of individual variability, both in humans and laboratory animals [17,27,31]. Psychostimulants activate the mesocorticolimbic dopamine system and individual vulnerability to their effects may reflect a given predisposition to dopaminergic psychosis, such as observed in drug addiction, schizophrenia and psychotic depression. Knowledge of factors that enhance vulnerability to psychostimulants will therefore greatly increase our insight in the neurobiology of dopaminergic psychopathologies.

The existence of marked strain differences in responsiveness to drugs such as amphetamine and cocaine has demonstrated that genetic traits contribute to variations in psychostimulant vulnerability. Two inbred mouse strains that have been used frequently to study the psychopharmacology of dopamine are the C57BL/6 and DBA/2 strains. These strains display profound differences in the anatomy and functioning of the mesocorticolimbic dopamine system and in behavioural responsiveness to dopaminergic agonists and addictive drugs (reviewed in [38]). Compared to DBA/2 mice, C57BL/6 mice are more sensitive to amphetamine-induced locomotion and reward and display higher drug-induced dopamine outflow in the nucleus accumbens [6,11,49,50,53]. Paradoxically, while C57BL/6 mice are also more vulnerable to the rewarding effects of cocaine, they appear less sensitive to cocaine-induced locomotion [28,47]. Robust differences between the two

Abbreviations: ACTH, adrenocorticotrophic hormone; ADX, adrenalectomy; ANOVA, analysis of variance; COC, cocaine; HPA-axis, hypothalamus–pituitary–adrenal-axis; RIA, radio-immuno-assay; SAL, saline; SHAM, Sham surgery

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strains have also been reported for behavioural sensitisation to repeatedly administered psychostimulants, although the magnitude and direction thereof appears to be highly dependent on the design of the sensitisation paradigm [2,6,28,40].

Interestingly, the strain differences in dopaminergic transmission and sensitivity to the rewarding properties of psychostimulants are not stable, but can change under the influence of environmental challenges, pointing towards a role for the neuroendocrine stress system in psychostimulant vulnerability [10,11,39,51]. Indeed, a wealth of data suggests that stress modulates behavioural and neurochemical responses to psychostimulants and other addictive drugs [18,24,34,35,45].

Stressful stimuli, either physical or mental, induce concomitant activation of the hypothalamus–pituitary–adrenal axis (HPA-axis) and the sympathetic nervous system resulting in release of glucocorticoid hormones and epinephrine from the adrenal glands [13]. Glucocorticoids in particular, have been shown to modulate transmission in the mesocorticolimbic dopamine system and to facilitate behavioural responses to psychostimulants such as locomotor activity, behavioural sensitisation, self-administration and relapse (reviewed in: [25]). Furthermore, corticosterone in the range of stress-induced levels has reinforcing potential and stress can, like drugs of abuse, increase strength of excitatory synapses on midbrain dopaminergic neurons [33,42]. Strong evidence indicates that the glucocorticoid–dopamine interactions are dependent on activation of the glucocorticoid receptor that is widely distributed throughout the brain and is expressed by the majority of the midbrain dopaminergic neurons [12,14,15,19,22,41,42].

Taken together, these data suggest that variations in HPA-axis responsiveness to stress may contribute to individual differences in psychostimulant vulnerability, as was elegantly addressed by Piazza et al. [36]. In this respect, laboratory mouse or rat strains with differential stress responsivity provide a valuable tool to study the interaction between the neuroendocrine stress system and the mesocorticolimbic dopamine circuit. With respect to the C57BL/6 and DBA/2 inbred strains, few studies have addressed differences in HPA-axis activation and findings are contradictory. In one study, C57BL/6 mice displayed higher peak corticosterone levels in response to novelty, which is in line with our findings (S. Dalm, personal communication), but contradictory to two reports using other stressors and experimental designs [7,23,46]. In addition, there may be differences between these strains in psychostimulant-induced HPA-axis activation, but this has to our knowledge not been reported yet. Differences in basal, stress- or psychostimulant-induced glucocorticoid release may however play a prominent role in the observed strain differences in psychostimulant sensitivity.

The present study was designed to test the hypothesis that adrenal stress hormones contribute to strain differences in cocaine sensitivity. The C57BL/6 and DBA/2 mouse strains were used as model for genetic differences in dopamine and HPA-axis function. We have measured behavioural sensitisation to the locomotor stimulant effect of cocaine and, in parallel, corticosterone responses to single and repeated cocaine exposure. In order to show involvement of the adrenal, we have tested whether strain differences persist when the adrenal is surgically removed (adrenalectomy: 'ADX') prior to the onset of drug treatment.

2. Methods

2.1. Animals

Male C57BL/6 Rj (C57BL/6) and DBA/2 Rj (DBA/2) mice were obtained from Janvier (Le Genest Saint Isle, France) and received in the animal facility at the age of 8 weeks. Mice were housed in groups of four of the same strain in perspex cages (35 cm × 19 cm × 14 cm) with food and water available *ad libitum* at a 12 h light–dark cycle (lights on: 7 am) in a temperature (21 ± 1 °C) and humidity (55 ± 5%) controlled room. Surgery was performed 2 weeks after arrival in the animal facility. Animals were briefly handled in the week before surgery and otherwise left undisturbed. Animal experiments were approved by the local Committee for Animal Health, Ethics and Research of Leiden University. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

2.2. Experimental design

The study consisted of eight experimental groups. Per mouse strain (C57BL/6 and DBA/2) animals were either SHAM operated (SHAM) or adrenalectomised (ADX). Each surgical group was subdivided into a cocaine (COC) and a saline (SAL) group, indicating the treatment given during the treatment period of the sensitisation paradigm. Each experimental group consisted of 9–12 animals.

2.3. Surgery

Animals were individually housed 1 day prior to surgery. The cages were transported to the operating room on the morning of the surgery where mice were allowed to recover from transportation for 2 h. Inhalation anaesthesia consisted of a mixture of isoflurane (3 l/min), N_2O (0.8 l/min) and O_2 (0.4 l/min). During surgery mice were placed on a heat-pad (37 °C). The skin on the back was shaved and disinfected and an incision of approximately 1 cm was made above and parallel to the spinal cord. Through a small opening in the muscle layer left and right of the spinal cord the adrenals were removed from the surrounding fat tissue. The skin was closed using a simple running suture. SHAM animals were treated similarly with the exception of the actual removal of the adrenals. Mice were kept individually housed for 24 h following surgery after which they were housed two animals per cage of similar surgery and strain. After surgery, all animals were given free access to 0.9% NaCl in addition to normal drinking water. The sensitisation paradigm was started following a recovery period of 1 week.

2.4. Drugs

Cocaine hydrochloride (BUFA Pharmaceuticals B.V., Uitgeest, The Netherlands) was dissolved in sterile saline, stored in aliquots at −20 °C and defrosted at the day of administration. Cocaine (room temperature) was administered intraperitoneally (i.p.) in a volume of 200 μl/25 g bodyweight. Control groups received an equal volume of saline. From the start of the sensitisation paradigm, animals were weighed once every 2 days and the injection volumes were adjusted accordingly.

2.5. Sensitisation paradigm

One day prior to the first drug administration and thus the first behavioural test, animals were individually housed and kept single housed for the remainder of the experiment.

The sensitisation paradigm consisted of a treatment phase (days 1–9), a withdrawal interval (days 10–14), a saline challenge (day 14) and a cocaine challenge (day 15). The treatment phase consisted of i.p. injections of 15.0 mg/kg cocaine (COC) or saline (SAL) on nine consecutive days and locomotion was measured on days 1 (first administration) and 9 (last administration). On days 2–8 animals received the injections in the home cage. The treatment period was followed by a withdrawal interval of 5-days (no treatment). On the last day of