Sustained Impairment of α_{2A}-Adrenergic Autoreceptor Signaling Mediates Neurochemical and Behavioral Sensitization to Amphetamine

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Background: In rodents, drugs of abuse induce locomotor hyperactivity, and repeating injections enhance this response. This effect, called behavioral sensitization, persists months after the last administration. It has been shown that behavioral sensitization to amphetamine develops parallel to an increased release of norepinephrine (NE) in the prefrontal cortex (PFC).

Methods: Rats and mice were repeatedly treated with amphetamine (1 or 2 mg/kg intraperitoneally, respectively) to obtain sensitized animals. The NE release in the PFC was measured by microdialysis in freely moving mice ($n = 55$). Activity of locus coeruleus (LC) noradrenergic neurons was determined in anaesthetized rats ($n = 15$) by in vivo extracellular electrophysiology. The α_{2A}-adrenergic autoreceptor (α_{2A}-AR) expression was assessed by autoradiography on brain slices, and Gαi proteins expression was measured by western blot analysis of LC punches.

Results: In sensitized rats LC neurons had a higher spontaneous firing rate, and clonidine—an α_{2A}-adrenergic agonist—inhibited LC neuronal firing less efficiently than in control animals. Clonidine also induced lower levels of NE release in the PFC of sensitized mice. This desensitization was maintained by a lower density of Gαi1 and Gαi2 proteins in the LC of sensitized mice rather than weaker α_{2A}-AR expression. Behavioral sensitization was facilitated by α_{2A}-AR antagonist, efaroxan, during amphetamine injections and abolished by clonidine treatment.

Conclusions: Our data indicate that noradrenergic inhibitory feedback is impaired for at least 1 month in rats and mice repeatedly treated with amphetamine. This work highlights the key role of noradrenergic autoreceptor signaling in the persistent modifications induced by repeated amphetamine administration.

Key Words: Gαi proteins, in vivo microdialysis, locus coeruleus cell recording, neurochemical sensitization, norepinephrine, psychostimulants

The development of effective treatments against drug addiction requires deeper knowledge of the long-term neurobiological modifications induced by chronic exposure to frequently abused drugs. Amphetamine is a psychostimulant that is widely abused and has long-lasting effects on the central nervous system. Its consumption disrupts the social and professional life of users who become addicted, and relapse is common, even after prolonged abstinence.

In rodents, locomotor responses to both psychostimulants and opiates are progressively and persistently enhanced by repeated exposure. This phenomenon, called behavioral sensitization, has been shown to be a suitable model for studying the modifications induced by drugs of abuse. Behavioral sensitization is long-lasting, specific to addictive compounds, and is thought to play a key role in the development of drug-seeking and drug-taking behaviors and in cue-induced relapse (1,2).

We previously showed, in C57BL/6J mice, that repeated injections of amphetamine led not only to behavioral sensitization but also to a neurochemical sensitization of noradrenergic and serotonergic neurons that persisted for at least 1 month after withdrawal. Similar findings have been reported for repeated exposure to most commonly abused drugs, including cocaine, morphine, ethanol, and nicotine in presence of monoamine oxidase inhibitors (3–5). After repeated injections, an amphetamine challenge induces a large increase in cortical extracellular norepinephrine (NE) levels that is correlated with a potentiation of drug-induced increases in locomotor activity.

In this study, we focused on the mechanisms underlying the maintenance of this long-term noradrenergic hyperreactivity. There are at least two possible non-mutually exclusive explanations for this sensitization: either amphetamine enhances the excitatory input to noradrenergic neurons or the increase in reactivity is due to disinhibition of noradrenergic transmission as a result of impaired inhibitory feedback. Initial experiments, presented here, are consistent with sensitization due to the disinhibition of noradrenergic transmission.

The pontine nucleus locus coeruleus (LC) is the only source of NE in the cerebral cortex (6). The neuronal activity of LC cells and NE release in target regions are subject to inhibitory control via α_{2A}-adrenergic autoreceptors (α_{2A}-AR). These receptors are coupled to Gαi/o proteins, and agonist stimulation leads to inhibition of the cyclic adenosine monophosphate pathway and a hyperpolarization of the noradrenergic cells through the activation of potassium ion conductance (7–9). There is evidence to suggest that α_{2A}-AR are involved in amphetamine-induced behavioral sensitization in mice. In

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fact, a study has shown that mice lacking the α_{2A}-adrenergic receptors exhibit an unclear locomotor sensitization to amphetamine (10). However, because these mutant mice were hyperreactive to an acute injection of amphetamine and are sensitized after saline treatment, no final conclusion about amphetamine behavioral sensitization could be drawn. Moreover, the implication of these receptors on neurochemical and behavioral effects of amphetamine repeated treatments as well as persistent molecular mechanisms implicated in these modifications have not been investigated in wild-type mice.

In this study, we investigated possible changes to α_{2A}-AR or their intracellular signaling in amphetamine-sensitized mice, to unravel the mechanism(s) underlying behavioral and neurochemical sensitizations. We first investigated whether the noradrenergic hyperreactivity observed in sensitized mice could be reproduced in naive mice by a blockade of noradrenergic autoreceptors. We then determined the spontaneous firing rate of LC cells and their sensitivity to the α_{2A}-AR agonist clonidine in animals previously sensitized to amphetamine and compared the results obtained with those for control animals. Rats were used in the LC recording experiments, to ensure reliable single-unit recording from a nucleus containing only 1500 cells, because in vivo recordings of LC neurons in mice and rats are similar (11). We carried out in vivo microdialysis to assess the ability of clonidine to decrease extracellular basal NE levels in the prefrontal cortex (PFC) of naive and sensitized mice. The density of α_{2A}-AR was determined by autoradiography, and the amount of Gαi/o protein available to mediate the molecular effects of these receptors was analyzed by western blotting in sensitized and naive mice. Finally, we analyzed the effects of autoreceptor stimulation or blockade on the development of amphetamine-induced behavioral sensitization.

Methods and Materials

Animals

Male C57BL/6J inbred mice (26 to 32 g) were purchased from Charles River (Wilmington, Massachusetts). Male Sprague-Dawley rats (Charles River) were 2 to 4 months old. Both mice and rats were housed with food and water supplied ad libitum and a 12-hour light/dark cycle (lights on at 7:30 AM). They were habituated to the laboratory regime for 1 week before the experiments began. Animals were treated in accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health and the European Community Council Directive 86/609/EEC.

Drugs

D-amphetamine sulfate, clonidine hydrochloride, 2-ethyl-2-(imidazolin-2-yl)-2,3-dihydrobenzofuran hydrochloride (efaroxan hydrochloride) were dissolved in saline. Drugs were purchased from Sigma Aldrich (L'isles d'Abeau Chesnes, France), with the exception of clonidine, which was obtained from ICN BIomedicals (Orsay, France). Drugs were injected intraperitoneally (IP) unless otherwise indicated. Doses are expressed as salts. For the experiments in mice, D-amphetamine sulfate was administered at a dose of 2 mg/kg (3). For locomotor activity measurements, clonidine was administered at a dose of .2 mg/kg, because it was the lowest dose blocking the locomotor response to 2 mg/kg amphetamine in test experiments. For microdialysis experiments, clonidine was administered at doses of 40 μg/kg subcutaneously (12). Efaroxan was always administered at a dose of 2.5 mg/kg.

For electrophysiological recordings in rats, animals were sensitized by 1 mg/kg amphetamine administrations, and reactivity of LC cells was tested with 40 μg/kg clonidine (13).

Timeline	Groups			
Daily injections (4 consecutive days)	Sal+ sal	Sal + amph	(Clo or Efa) + amph	(Clo or Efa) + sal
Day 25 Challenge	Amph			

Figure 1. Protocol of behavioral sensitization to amphetamine in presence of clonidine or efaroxan. Illustration of the protocol of behavioral sensitization to amphetamine (amph) in the presence of clonidine (clo) (.2mg/kg intraperitoneally) or efaroxan (efa) (2.5mg/kg intraperitoneally). sal, saline.

Locomotor Activity

Mice. Mice were introduced into a circular corridor (Imetronic, Pessac, France). Locomotor activity was counted when animals traveled along one quarter and expressed as the number of 1/4 turns/5 min. Spontaneous activity was recorded for 120 min, and the mice then received injection with amphetamine, efaroxan plus amphetamine, or clonidine plus amphetamine (Figure 1). Clonidine was administered twice (30 min before and 1 hour after amphetamine injection) to maintain its concentration during the effects of amphetamine. Only mice displaying locomotor activity superior to 120 1/4 turns/5 min were considered as sensitized.

Rats. We used the same protocol for rats but with an appropriately scaled-up apparatus (Imetronic, Bordeaux, France). Rats received one injection of amphetamine or saline on each of 4 consecutive days and an additional one after 4 days of withdrawal. Activity was measured for 2 hours before injection and 3 hours after injection. Only rats displaying a locomotor activity of 300% of the first injection locomotor response were retained 1 month later for the electrophysiological analysis of sensitized animals.

Electrophysiological Recordings

Electrophysiological recordings were carried out 1 month after the last amphetamine injection. Rats were anesthetized with urethane (1.5 g/kg), and the body temperature was maintained at 37°C. The animal was fixed in a stereotaxic apparatus, with the head at an angle of 14° below the horizontal plane, to permit access to the LC. Electrodes were inserted at the following coordinates: 2 mm anterior to bregma, .5 mm lateral to the midline for frontal, and 4 mm posterior to lambda, 1.2 mm lateral to the midline for LC recording. Characterization of LC units and specific details concerning extracellular recordings are detailed in Supplement 1.

Histology

Direct current (9 volts) was passed through the electrode for 5 sec to create a small lesion at the tip. Rats were perfused intracardially with saline, followed by 10% formalin. Brains were removed, stored in formalin for 1 week, and then cut into 60-μm sections and stained with cresyl violet.

Microdialysis Surgery

Mice were anesthetized with sodium pentobarbital (60 mg/kg; Sanofi Santé Animale, France). A unilateral permanent cannula (CMA/7 guide cannula; Phymep, Paris, France) was placed at the edge of the PFC. The coordinates for the guide cannula tips were: anteroposterior, +2.6 mm relative to bregma; mediolateral, +.5 mm; dorsoventral, 0 from dura (14). After surgery, mice were placed in individual cages and allowed to recover for at least 4 days.

Microdialysis Experiments

On the day of the experiment, the microdialysis probe was inserted into the PFC (membrane length: 2 mm; diameter: 24 mm;