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Short communication

Opposite effects of stress on dopamine release in the limbic system of drug-naive and chronically amphetamine-treated rats

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Abstract

The effects of repeated amphetamine administration on stress-induced dopamine release in the ventral striatum were examined in male Wistar rats treated with D-amphetamine (1.5 mg/kg; i.p./injection) or saline at 12 h intervals for 14 days. After 12 h as well as 7 days of amphetamine withdrawal, dopamine release was monitored by transverse microdialysis under basal conditions and during exposure to 60 min of restraint stress. Basal dopamine release was significantly suppressed relative to saline-pretreated controls after both 12 h and 7 days of amphetamine withdrawal. In control rats, restraint stress resulted in significantly increased dopamine efflux. In contrast, exposure to this stressor was associated with a significant suppression of dopamine release in rats chronically exposed to amphetamine. This effect was observed at both post-amphetamine test points. The results suggest that chronic amphetamine impairs the dopaminergic response to stress and that this dopaminergic deficit may play a role in stress-induced drug-seeking behavior and relapse. © 1997 Elsevier Science B.V.

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1. Introduction

High rates of relapse after abstinence present a considerable problem in the treatment of drug abuse and dependence. Among the variety of stimulus conditions that appear to play a role in relapse, clinical studies have drawn attention to the significance of stress and negative mood states. For instance, stressful life events can increase drug use or trigger episodes of relapse in dependent drug abuse patients (e.g., Krueger, 1981; Marlatt, 1985; Wallace, 1989; Brown et al., 1995). It has also been suggested that a link exists between responsiveness to stress and vulnerability to drug dependence, and that susceptible individuals use drugs as a means of coping with stress (Khantzian et al., 1974; Tarter, 1988).

Data from animal models of drug self-administration indicate that stress can increase drug-seeking behavior and enhance the rewarding effects of drugs of abuse. Physical, social and emotional stressors have been shown to increase self-administration of psychostimulants, ethanol and opiate drugs or to facilitate the acquisition of drug self-administration (e.g., Mollenauer et al., 1993; Ramsey and Van Ree, 1993; Shaham and Stewart, 1994; Goeders and Guerin, 1994; Haney et al., 1995). Of particular importance with regard to a role of stress in relapse is the finding that immobilization and footshock stress can reinstate heroin-seeking behavior in drug-free animals (Shaham and Stewart, 1995). Interestingly, stress and drugs of abuse share the property of increasing extracellular dopamine levels in limbic brain regions (Imperato et al., 1992a; Di Chiara and Imperato, 1988; Shaham and Stewart, 1995), suggesting that activation of dopaminergic neurotransmission may be involved in the ability of stressful stimuli to elicit drug-seeking behavior or to increase drug intake. On the other hand, deficits in extracellular dopamine concentrations observed after chronic exposure to various drugs of abuse have also been implicated in drug-seeking behavior, particularly in the resumption of drug-taking behavior by dependent subjects during abstinence and withdrawal (e.g., Ros-

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setti et al., 1992; Imperato et al., 1992b; Weiss et al., 1996). Thus, important interactions may exist between the effects of stress and chronic drug treatments on mesolimbic dopamine activity that may contribute to enhanced drug-seeking behavior and relapse. The present study was designed to explore the neurobiological basis of interactions between stress and chronic drug exposure by examining the effects of restraint stress on dopamine release in the ventral striatum before and after chronic amphetamine administration and withdrawal.

2. Materials and methods

Male Wistar rats weighing 200–250 g were injected with D-amphetamine (1.5 mg/kg, i.p., b.i.d.) or saline (1.5 ml/kg) at 12 h intervals for 14 days. On the 13th day of treatment the animals were surgically implanted under halothane anesthesia with a transverse dialysis probe in the ventral striatum, which was perfused with Ringer solution at a rate of 2 μl/min according to the methods of Imperato et al. (1992a). The experiments started 24 h after implantation of the dialysis probes. Rats were exposed to 60 min of immobilization stress at 12 h and, again, 7 days after the last chronic injection. All tests were conducted at the same time of day between 09.30 and 05.00 during the light phase of the rats' light/dark cycle. Extracellular dopamine concentrations were measured for 60 min before, during and 30 min after restraint on each occasion. Microdialysates were assayed for dopamine content by reverse-phase high performance liquid chromatography with electrochemical detection. The detection limit for dopamine ranged between 0.002 and 0.005 pM per 20 μl sample.

3. Results

Confirming previous observations (Imperato et al., 1992a), restraint stress significantly increased dopamine efflux in the ventral striatum of drug-naive control rats to a maximum of approximately 50% above baseline within 10 min after the onset of restraint ($F(9,27) = 15.35$, $P < 0.0001$; two-way within-subjects ANOVA). Dopamine concentrations in these animals returned to baseline in about 60 min with a second modest increase (about 30%) within 10 min after discontinuation of restraint (Fig. 1). The stress-induced and post-stress increases in dopamine release (expressed as percent of baseline) in drug-naive control rats were nearly identical during the 12 h and 7 day post-treatment tests ($F(1,3) = 2.04$, NS). Differences in dopamine efflux from basal levels at individual time points during the experimental session were therefore determined from the pooled data of the 12 h and 7 day tests. These analyses confirmed significant increases in dopamine release during the 20–40 min ($P < 0.01$; restraint stress) and the 70–90 min ($P < 0.05$; post-stress) sampling periods (Newman–Keuls after significant main effect of 'time' shown above).

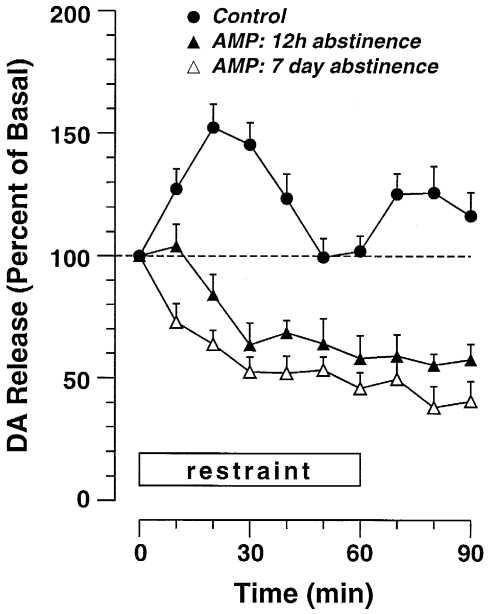

Fig. 1. Effect of restraint stress on extracellular dopamine concentrations in the ventral striatum of control rats and rats chronically treated with D-amphetamine and withdrawn from the drug for 12 h (AMP: 12 h abstinence) or 7 days (AMP: 7 day abstinence). Data are expressed as the mean (±S.E.M.) percent variation from baseline, defined as the average dopamine concentration across three samples collected during the 60 min preceding restraint. The profile of dopamine release during the stress and post-stress phases in drug-naive control rats were similar during the 12 h and 7 day post-treatment tests. Therefore, the data obtained at the two test times were pooled for the purpose of illustration. Dotted line represents baseline dopamine release. For statistical comparisons, see text.

In contrast to drug-naive animals, rats chronically pretreated with amphetamine showed significant 40% (12 h abstinence group: $F(9,27) = 6.51$, $P < 0.0001$) and 50% (7 day abstinence group: $F(9,27) = 8.69$, $P < 0.0001$) reductions in extracellular dopamine concentrations during restraint which persisted throughout the remaining 30 min sampling period following cessation of the stress exposure (simple effects after confirmation of overall differences in dopamine release from baseline by two-way within-subjects ANOVA: $F(3,9) = 11.46$, $P < 0.0001$). Basal dopamine concentrations, defined as the mean value across three consecutive samples collected over a 60 min period before restraint, were significantly reduced in the amphetamine-treated rats both 12 h and 7 days after the final drug injection. The mean (±S.E.M.) basal dialysate dopamine levels (pmol/10 min in 20 μl samples; not corrected for recovery) were 0.3 ± 0.008 pmol in saline-treated control versus 0.21 ± 0.009 pmol in amphetamine-treated rats at the 12 h test time ($F(1,8) = 118.15$; $P < 0.0001$) and 0.22 ± 0.01 pmol (saline) versus 0.19 ± 0.009 pmol (amphetamine) 7 days after termination of chronic injections (($F(1,8) = 7.13$; $P < 0.05$; simple effects analy-

ses after significant main effect of 'amphetamine' in overall mixed-factorial ANOVA: $F(1,6) = 55.8$; $P < 0.0005$). Thus, relative to baseline dopamine release in saline-treated controls, the actual reduction in dopamine concentrations after restraint stress in chronic amphetamine-treated rats reached approximately 60% on both testing occasions.

4. Discussion

The results confirm that chronic pretreatment with amphetamine reduces extracellular dopamine concentrations in the ventral striatum (Rossetti et al., 1992). More importantly, the results show that the stimulation of dopamine release in the ventral striatum in response to restraint stress that is typically observed in untreated animals (Imperato et al., 1992a) can no longer be observed after chronic amphetamine treatment. In fact, restraint stress in chronic amphetamine-exposed rats produced a persistent reduction in extracellular dopamine concentration below baseline levels that were already lowered by the chronic drug treatment. These observations are not in conflict with the results of Hamamura and Fibiger (1993) who reported that repeated amphetamine administration enhances stress-induced dopamine release in the prefrontal cortex since reciprocal changes in the prefrontal cortex and ventral striatum have been observed under different experimental conditions and dopamine in the prefrontal cortex has been suggested to exert an inhibitory control over subcortical dopaminergic transmission (Le Moal and Simon, 1991).

The effects observed here cannot be attributed to artifacts associated with the continuous microdialysis procedure since in saline pretreated controls, the effects of stress on dopamine release were nearly identical on both testing occasions. Moreover, the validity of the present procedures has been carefully confirmed in previous work showing that increases in extracellular concentrations of dopamine and its major metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, after peripheral administration of haloperidol remain constant throughout a 10-day period (Imperato et al., 1992b). Finally, in a small group of control animals included in the present experiment ($n = 2$), haloperidol (0.5 mg/kg; i.p.) produced identical 90–100% increases in dopamine release over baseline, 1, 7 and 14 days after implantation of the microdialysis probe (data not shown).

The reduction in basal extracellular dopamine concentrations in the ventral striatum at both the 12 h and 7 day post-amphetamine abstinence points is consistent with previous findings (Rossetti et al., 1992). Deficits in extracellular dopamine levels have also been observed after termination of chronic treatment with other drugs of abuse including cocaine, morphine and ethanol (Imperato et al., 1992b; Rossetti et al., 1992; Weiss et al., 1996). Such neurochemical deficiencies have been implicated in symptoms of dysphoria and negative affect during withdrawal from chronic drug treatments and, according to neuroadaptation theories (e.g., Koob and Bloom, 1988) may ultimately underlie the motivation to resume drug-taking behavior in dependent subjects. The present data suggest that certain forms of stress may exacerbate the neurochemical consequences of amphetamine withdrawal by further lowering extracellular dopamine levels and, thereby, perhaps contribute to the resumption of drug-seeking behavior and increased likelihood of relapse associated with stress. Since this effect was not confined to acute abstinence but was still observed at the same magnitude 7 days post-amphetamine, it may play a role in vulnerability to relapse over a prolonged abstinence period.

Whether the restraint-stress induced decrease in dopamine efflux can be generalized to other drugs of abuse remains to be determined. Footshock stress did not alter dopamine release in the nucleus accumbens in rats after repeated cocaine pretreatment (Kalivas and Duffy, 1989) and increased dopamine release in rats previously self-administering heroin (Shaham and Stewart, 1995). It is unclear whether the differences between these earlier and the present results can be explained by differences in the long-term pharmacological actions of amphetamine, cocaine and heroin alone, or whether differences in the duration and dosage of the drug treatment are a determining factor. However, if an impairment in the dopaminergic response to stress is a common feature associated with withdrawal from drugs of abuse, particularly after aggressive or dependence-inducing treatment regimens, this phenomenon may play an important role in the relapse to drug seeking behavior in general.

Of importance with regard to withdrawal-associated affective changes that may contribute to drug-seeking behavior is also the observation that chronic amphetamine treatment abolished the dopaminergic response both to the exposure to restraint stress as well as the termination of the stressful experience. The biphasic increase in dopamine release in control rats after both restraint and release from immobilization in controls confirms earlier observations and has been interpreted to reflect emotional arousal produced by both aversive and rewarding stimuli (Imperato et al., 1992a). Interestingly, this work has also shown that the dopaminergic response to the onset, but not termination of restraint stress habituates and diminishes with repeated exposure. Since, as opposed to restraint, the release from immobilization may be considered a rewarding event, these findings suggest that mesolimbic dopaminergic activation by aversive and pleasurable experiences involves separate neural systems that adapt differently to repeated exposure (Imperato et al., 1992a). While the nature of these neural interactions is, at present, not well understood, the failure in the present experiment to observe an activation of dopamine release in response to either the onset or the termination of restraint stress suggests that the neural mechanisms involved in the dopaminergic response to aversive and rewarding stimuli are both compromised after

chronic amphetamine. For example, the lack of dopaminergic activation after removal from restraint is consistent with brain stimulation reward deficits that are associated with amphetamine withdrawal (e.g., Leith and Barrett, 1976) and points toward a dysfunction in brain reward systems. It is possible that the persistent suppression in dopamine release after both stress and removal from stress during amphetamine abstinence reflects a disruption of mechanisms that regulate affective homeostasis, leading to an impairment in the ability to cope with stress or affective challenges. Such defects may have important implications for emotional states such as depression, helplessness and despair. More importantly, since it is known that with many drugs of abuse 70% to 80% of relapse takes place in situations of stress, conflict and social pressure (Marlatt, 1985), such defects may have an important role in the resumption of drug-taking behavior.

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