Adrenergic Hyperactivity and Metanephrine Excess in the Nucleus Accumbens After Prefrontocortical Dopamine Depletion

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Espejo, Emilio F. and Javier Miñano. Adrenergic hyperactivity and metanephrine excess in the nucleus accumbens after prefrontocortical dopamine depletion. J Neurophysiol 85: 1270–1274, 2001. Selective dopamine depletion within the medial prefrontal cortex in rats is known to enhance dopamine and norepinephrine levels in the nucleus accumbens and to induce characteristic behavioral disturbances. The present study was designed to determine levels of adrenaline, apart from dopamine and norepinephrine, and metabolites in the nucleus accumbens after prefrontocortical dopamine depletion. Prefrontocortical dopamine depletion was carried out by injecting 6-hydroxydopamine, and it was validated through: the emergence of behavioral disturbances such as amphetamine-induced stereotypies, spontaneous motor hyperactivity, and enhanced “anxiety-like” responses and through postmortem quantification of catecholamine levels by using high-performance liquid chromatography. The findings indicated that lesioned rats exhibited more oral stereotypies after amphetamine, were hyperlocomotive, and showed more pronounced anxiety-like behaviors than controls. Following prefrontocortical dopamine depletion, postmortem concentrations of dopamine and norepinephrine, along with the metabolites 3,4-dihydroxyphenylacetic acid and vanillylmandelic acid, were reliably enhanced in the nucleus accumbens as expected, and dopamine turnover was decreased. Furthermore the nucleus accumbens contained higher levels of adrenaline and its transmethylated metabolite metanephrine. To sum up, prefrontocortical dopamine depletion induces motor and emotional disturbances in rats and alters the neurochemical profile of the nucleus accumbens, not only inducing dopaminergic and noradrenergic hyperactivity but also leading to adrenaline and metanephrine excess.

INTRODUCTION

Dopamine (DA) depletion in the medial prefrontal cortex (mPFC) in rats is known to impair selective attention, to increase spontaneous motor activity, to induce behavioral stereotypes, and to alter the emotional state, e.g., inducing “anxiety-like” behaviors (Bubser and Koch 1994; Carter and Pycok 1980; Espejo 1997; Lacroix et al. 1998; Le Moal and Simon 1991). Selective depletion of DA within the medial prefrontal cortex of rats and monkeys has also been reported to enhance DA levels within the nucleus accumbens (Carter and Pycok 1978, 1980; Kolachana et al. 1995; Kurachi et al. 1995; Pycok et al. 1980; Roberts et al. 1994; Sokolowski and Salamone 1994), which has led to the hypothesis that behavioral disturbances are mostly caused by a hypodopaminergic prefrontal activity together with a reactively enhanced dopaminergic tone in the nucleus accumbens (Grace 1991).

Although studies on catecholamine levels within the nucleus accumbens have been centered on dopamine and norepinephrine (NE), which has been reported to be also enhanced after prefrontal DA lesion (Christie et al. 1986), other biogenic amines such as adrenaline (A) could be affected as well. This fact has been unexplored so far because it is known that adrenergic innervation of nucleus accumbens is sparse in rats (Saavedra et al. 1974; van der Gugten et al. 1976), although it is worth noting that adrenergic innervation of nucleus accumbens is known to be more dense in humans than in rats (Lew et al. 1977; Saavedra et al. 1974). Catecholaminergic changes in the nucleus accumbens are supposed to have important functional implications because this nucleus is considered as the main interface between limbic and motor systems, a key neural structure supporting the flow from “motivation” into “action” (Mogenson et al. 1980).

The objective of this study was to employ the prefrontocortical dopamine depletion model in rats to determine postmortem levels of all catecholamines, including adrenaline and metabolites in the nucleus accumbens. The prefrontocortical lesion was validated by the behavioral profile of lesioned rats, and postmortem high-performance liquid chromatography (HPLC) measurements.

METHODS

Animals and ethics

Male Wistar rats (275–325 g) from the breeding colony of the Faculty of Medicine of the University of Seville, Spain, were housed in the vivarium in groups of three. Laboratory temperature was kept at 22 ± 1°C, and a 12-h light-dark cycle (lights on at 08:00 h) was maintained throughout the experiment. Food (lab chow) and water were available ad lib. Experiments were performed in accordance with the European Communities Council Directive for the employment of laboratory animals (86/609/EEC).

Prefrontocortical lesion with 6-OHDA

Thirty minutes before 6-hydroxydopamine (6-OHDA) lesion, rats were injected with desipramine (15 mg/kg ip) to protect noradrenergic terminals from 6-OHDA toxicity. Then rats were anesthetized with...
Behavioral testing

Two weeks after the lesions, stereotypies were evaluated after injection of amphetamine sulfate (5 mg/kg ip, RBI). Thus immediately after amphetamine, rats were placed in a transparent chamber (50 × 24 × 24 cm), and four behavioral patterns were quantified (sniffing, licking, gnawing, walking), from 30 through 45 min postinjection. The occurrence of a given behavior, but not the actual frequency of each behavioral pattern, was recorded every 10 s to determine the repetition of a behavior.

Two days after the amphetamine test, “anxiety-like” behaviors were analyzed by using a 5-min light/dark test, an anxiety model. The apparatus consisted of a two-compartment rectangular box made up of Plexiglas, with one compartment painted white (30 × 48 × 34 cm high) and illuminated by a 60-W white incandescent bulb (900 lx) and the other compartment painted black (30 × 48 × 34 cm high), without illumination. The two compartments were connected by a small opening (9 × 8 cm). Rats were placed in the light compartment at the beginning of the test, and several behavioral parameters were quantified: time spent in light compartment, time spent in dark compartment, number of transitions from light to dark, number of transitions from dark to light, and number of stretched attend postures (SAP) from dark to light. Operational criterion for entry was whole body and four paws entry. Two days after the light/dark test, locomotor activity was evaluated. Each rat was placed in an open field (1 × 1 m, 25 × 25 cm each quadrant) for 10 min. Three locomotor parameters were quantified: distance traveled (cm), duration of mobile exploration (walking while sniffing), and speed of movements (cm/s). The rats’ behavior during the light/dark and open field tests was recorded on videotape. The tapes were later visualized, and the behavior was analyzed by keyboard entry to a computer programmed to perform statistical and ethnological analyses, with ethnological software developed by E. F. Espejo in the laboratory. Video tapes were scored “blind” by two highly trained observers (intra-rater reliability ≥0.9). All behavioral tests were carried out during the last phase of the light period (18:00 to 20:00 h).

Postmortem neurochemical analysis and statistics

Two days after the last behavioral test, all the rats were killed by decapitation, the brains were quickly removed and placed on ice, and bilateral medial prefrontal cortex and nucleus accumbens were immediately dissected, weighed, and frozen at −80°C. Later, tissue samples were homogenized in 0.5 ml of an ice-cold solution containing 0.4 M HClO₄, 0.5 M sodium acetate, and 0.5 M acetic acid centrifuged at 27,000 rpm for 60 min at 4°C. The supernatants were decanted and filtered through a 0.45 μm filter (Sartorius), and frozen at −80°C until HPLC assay. The electrochemical performance was based essentially on the method described by Saito et al. (1992). Aliquots (10 μl) of each sample were injected directly into the HPLC system (System Gold, Beckman), consisting of a solvent delivery pump with a pulse-dampener, an automatic sample injector (Carnegie Medicine) and an analytical C18 reverse-phase column (Ultrasphere 3-μm particle size, 75 × 4.6 mm ID, Beckman). The ESA model 5100 A Coulometric electrochemical detection system consisted of a model 5021 conditioning cell (detector setting +400 mV) followed in sequence by a model 5011 dual electrode analytical cell (cell 1, +100 mV; cell 2, −260 mV). The output signal from the final electrode was amplified by a 5100 A controller and relayed to an integrator (Model 106, Beckman). The detection limit of the system was 0.1 pg/μl (e.g., the detection limit for adrenaline was 1 fmol/μl).

The mobile phase for the separation of catecholamines and their metabolites was a mixture of 0.075 M Na₂HPO₄, 1.2 mM sodium heptanosulfonate, 0.097 mM EDTA, and 8% methanol (vol/vol) adjusted to pH 3.6. The buffer solution was filtered through a 0.45-μm membrane filter and degassed. The flow rate was set to 1.7 ml/min, and pressure was around 2000 psi. The mobile phase was recycled for 2 wk of continuous use before being replaced with fresh solution. The entire chromatographic system was run at ambient temperature. Peaks of biogenic amines and metabolites were identified by comparing the

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**TABLE 1.** Locomotor parameters in sham control and 6-OHDA-induced lesioned rats in the open field test (10 min)

<table>
<thead>
<tr>
<th>Group</th>
<th>Distance Traveled, cm</th>
<th>Mobile Exploration, s</th>
<th>Speed, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>4125 ± 351</td>
<td>303.3 ± 27</td>
<td>13.6 ± 1.7</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>5725 ± 325*</td>
<td>362.3 ± 21*</td>
<td>15.8 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. 6-OHDA, rats with prefrontocortical dopamine depletion induced by 6-hydroxydopamine (6-OHDA). *P < 0.05 vs. sham (Student’s t-test).

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FIG. 1. Stereotyped activities elicited by sham control and 6-hydroxydopamine (6-OHDA)-induced lesioned rats after amphetamine injection (5 mg/kg ip). Stereotype rating data are represented by the number of 10-s intervals in which each response was observed. Mean ± SE. * P < 0.05 vs. controls (Student’s t-test).
The content of monoamines was quantified in the mPFC by HPLC to validate the degree of DA depletion. In the nucleus accumbens, the contents of DA, NE, and A along with those of the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), vanillylmandelic acid (VMA), and metanephrine (MET) were quantified. Dopamine turnover was estimated by the DOPAC/DA ratio (calculated in pmols). Neurochemical and behavioral results were analyzed by using the Student’s t-test for comparison of two independent groups. Since size populations were small, the data were logarithmically transformed (log [x]) if variance was not homogeneous.

**RESULTS**

**Behavioral study**

AMPHETAMINE-INDUCED STEREOTYPIES. Student’s t-test revealed that lesioned rats displayed a significantly higher number of “licking” than controls ($P < 0.05$), and no differences were found between the remainder patterns, as shown in Fig. 1.

OPEN-FIELD TEST. As shown in Table 1, significant differences were obtained for distance traveled ($t = 2.5, P < 0.05$) and duration of mobile exploration ($t = 2.6, P < 0.05$) but not for speed of movements. These results indicate that there was a locomotor hyperactivity in lesioned rats affecting the time spent on walking and exploring the environment, but the speed of movements was not altered.

LIGHT/DARK TEST. As shown in Table 2, 6-OHDA treatment induced a significant reduction versus controls in time spent on light ($t = 2.3, P < 0.05$) and number of SAP from dark to light ($t = 2.4; P < 0.05$). Time spent on dark compartment was significantly enhanced after DA depletion ($t = 2.3, P < 0.05$).

**DISCUSSION**

Behavioral study

Prefrontocortical dopamine depletion induced the emergence of licking stereotypy after amphetamine, a drug that causes dopamine release from dopaminergic neurons and blocks dopamine transporter leading to enhancement of extraneuronal dopamine levels (Schwarting and Huston 1996). Oral stereotypies such as licking behavior have been correlated to hyperdopaminergic activity within either the shell territory of the nucleus accumbens (Cools et al. 1993; Prinssen et al. 1994) or the ventrolateral part of the dorsal striatum (Jicha and Salamone 1991; Kelley et al. 1988). Hence the findings of the present study can be accounted for by a stronger enhancement of dopamine release after amphetamine, either within the shell of the nucleus accumbens or the ventrolateral part of the dorsal striatum, suggesting that normal dopaminergic activity within these areas is affected after prefrontocortical dopamine depletion.

The spontaneous locomotor profile of lesioned rats was altered after prefrontocortical dopamine depletion. Lesioned rats showed an enhanced duration of mobile exploration of the new environment, although the mean speed of movements was not reliably enhanced. These results support previous findings reporting that there is spontaneous motor hyperactivity after prefrontocortical dopamine depletion (Carter and Pycock 1980) and indicate that prefrontal lesion affects motor initiation rather than motor execution (Hauber et al. 1994). Motor hyperactivity seems to be linked to the striatal hyperdopaminergic tone as well since hyperlocomotion is a common feature after drugs that enhance dopamine release within the striatum.

Finally, the anxiety level was enhanced after prefrontocortical dopamine depletion, as indicated by a reduction in time spent on light in the light/dark test.

Table 2. Behavioral parameters in sham control and 6-OHDA-induced lesioned rats in the light/dark test (5 min)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time on Light, s</th>
<th>Time on Dark, s</th>
<th>SAP From Dark to Light</th>
<th>Dark to Light Transitions</th>
<th>Light to Dark Transitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>57 ± 14</td>
<td>243 ± 14</td>
<td>11.6 ± 2.2</td>
<td>1.3 ± 0.4</td>
<td>2.1 ± 0.32</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>23.1 ± 6.2*</td>
<td>276.9 ± 6.1*</td>
<td>6.1 ± 0.6*</td>
<td>0.8 ± 0.2</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. 6-OHDA, rats with medialprefrontal cortex (mPFC) dopamine (DA) depletion induced by 6-OHDA; SAP, stretched atten posture. * $P < 0.05$ vs. controls (Student’s t-test).

Table 3. Cathecolamine concentrations within the medial prefrontal cortex in sham control and 6-OHDA-induced lesioned rats (pg/mg wet weight of tissue)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Group</th>
<th>DA</th>
<th>NE</th>
<th>A</th>
<th>n.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial prefrontal</td>
<td>Sham</td>
<td>121.8 ± 5.5</td>
<td>394.9 ± 41.4</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>20.9 ± 2.2**</td>
<td>305.6 ± 35.5*</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. NE, norepinephrine; A, adrenaline; n.d.: non-detectable. * $P < 0.05$, ** $P < 0.01$ vs. sham group (Student’s t-test).
spend in the light compartment and the number of SAP from dark to light, along with the enhancement of time spent in the dark compartment (Chauloff et al. 1997; Merlo-Pich and Samanin 1986). This result is consistent with a similar finding of our group using rats subjected to prefrontocortical dopamine depletion and then placed on the elevated plus-maze, another anxiety model (Espejo 1997). Since the prefrontal cortex is known to be involved in attention and cognitive processes, all these findings indicate that prefrontocortical dopamine depletion led to an abnormal prefrontocortical activity manifested as inadequate exploratory activity in a challenging situation, likely due to either low attention level or disruption of cognitive processes, with a strong anxiety component (Herman et al. 1982; Lavielle et al. 1979; Le Moal and Simon 1991). This finding confirms that, apart from motor disturbances, emotional responses are also altered after prefrontocortical dopamine depletion.

Neurochemical study

Neurochemical results indicated that DA within the mPFC was strongly depleted after 6-OHDA-induced lesion and that prefrontal norepinephrine level was reduced to a lesser extent. Adrenaline was undetectable, suggesting that the adrenergic innervation of the medial prefrontal cortex appears to be absent, in accordance with Scatton and Bartholini (1976). Dopamine depletion within the mPFC was correlated with a significant increase in accumbal dopamine content, in accordance with other authors (Carter and Pycock 1978, 1980; Kolachana et al. 1995; Kurachi et al. 1995; Pycock et al. 1980; Roberts et al. 1994; Sokolowski and Salamone 1994), although accumbal DA turnover was reduced after lesion. These findings could be interpreted as reflecting a decrease in firing of DA neurons projecting to nucleus accumbens after DA lesion of the mPFC in accordance with electrophysiological studies (Harden et al. 1998). The observed increases in activity of lesioned rats could be accounted for an enhanced phasic release of DA (Grace 1991), suggesting that a decrease in baseline DA levels would cause an augmented phasic DA response to behavioral demands or to amphetamine. The findings support the hypothesis that a hyperdopaminergic tone emerges in the nucleus accumbens after prefrontocortical dopamine depletion (Grace 1991).

Norepinephrine content in the nucleus accumbens was observed to be reliably enhanced (35.1%). This result is in accordance with others (Christie et al. 1986), and the increase of norepinephrine levels supports a functional role for this amine in this behavioral model (Oades et al. 1986; Svensson and Ahlenius 1982). Of note is that adrenaline levels were also reliably enhanced in lesioned rats (140%) along with the concentration of its metabolite metanephrine (50.9%). Although adrenaline content was very low in control rats in accordance with several authors (Saavedra et al. 1974; van der Gutgen et al. 1976), prefrontocortical dopamine depletion induced a reliable enhancement of this monoamine. These findings show, for the first time to our knowledge, that accumbal adrenaline concentration is enhanced and that there are accrued contents of metanephrine, its transmethylated metabolite, after prefrontocortical dopamine depletion. Finally, the enhancement of VMA levels can be accounted for by the increase in both norepinephrine and adrenaline, its parent monoamines. All these neurochemical facts surely play a functional role on prefrontocortical dopamine depleted rats. However, to establish the specific role of each monoamine and their receptors on the observed behavioral changes requires the use of specific drugs, such as receptor antagonists.

In this context, some authors have hypothesized that there is an hypodopaminergic prefrontal activity in human schizophrenia that is somehow related to a reactive hyperdopaminergic activity in subcortical areas such as the nucleus accumbens (Grace 1991). This inverse relationship between mesocortical and mesostriatal dopaminergic systems is supported by the results of the present study. Furthermore neurochemical changes observed in catecholamines and transmethylated metabolites fit well with the hypothesis of “chronic accumulation of amines and transmethylated metabolites” in schizophrenia, at least as far as the nucleus accumbens is concerned (Cadet and Kahler 1994; Heikkila and Cohen 1973; Willis and Armstrong 1998). Thus maintained brain monoamine excess is suspected to induce neurodegeneration through free radical and melanin production, and transmethylated product excess has been proposed to be “psychotoxic” (Cadet and Kahler 1994; Godal 1976; Smythies 1984; Trzeziak 1973; Willis and Armstrong 1998). It is worth noting that cerebrospinal metanephrine is a valid predictor of clinical response in schizophrenic patients, together with 3-hydroxykynurenine, a serotonin metabolite (Issa et al. 1994). Metanephrine was found to be strongly augmented in the rat model used in the present study, resembling changes observed in humans. This fact could be linked to an accumbal hyperadrenergic tone in schizophrenics. However, the validity of the prefrontocortical dopamine depletion model in rats to study some aspects of the pathophysiology of human schizophrenia is highly problematic and a matter of debate. Furthermore an almost complete depletion in the prefrontal cortex of rats can hardly mimic presumed “hypodopaminergic state” in human schizophrenia.

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### Table 4. Concentrations of catecholamines and metabolites within the nucleus accumbens after sham or 6-OHDA-induced lesion of the mPFC (pg/mg wet weight of tissue)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Group</th>
<th>DA (pg/mg)</th>
<th>DOPAC (pg/mg)</th>
<th>NE (pg/mg)</th>
<th>VMA (pg/mg)</th>
<th>A (pg/mg)</th>
<th>MET (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus</td>
<td>Sham</td>
<td>5779.8 ± 445.5</td>
<td>1090.8 ± 110.3</td>
<td>168.9 ± 11.5</td>
<td>147.4 ± 48.7</td>
<td>15 ± 3.1</td>
<td>73.6 ± 10.1</td>
</tr>
<tr>
<td>Accumbens</td>
<td>6-OHDA</td>
<td>1462.2 ± 1455.9**</td>
<td>1463.3 ± 150.6*</td>
<td>228.2 ± 21.2*</td>
<td>578.3 ± 87.1**</td>
<td>36 ± 4.1**</td>
<td>111.1 ± 10.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. DOPAC, 3,4-dihydroxyphenylacetic acid; VMA, vanillylmandelic acid; MET, metanephrine. *P < 0.05, **P < 0.01 vs. sham group (Student’s t-test).