Nitric oxide modulates the amphetamine effect on $[^3H]$glucose uptake in the brain of rats prenatally exposed to lead

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Abstract:
Glucose is the main source of energy for the central nervous system (CNS). In this study, we examined the effects of the psychostimulant amphetamine (AMPH) and the neuronal mediator nitric oxide (NO) on $[^3H]$glucose uptake in the brain of adult rats that had been prenatally exposed to lead. Lead [Pb(CH$_3$COO)$_2$·3H$_2$O; 250 ppm] was added to the drinking water of pregnant Wistar rats for the duration of pregnancy. On the day of parturition, lead was discontinued as an additive in the drinking water. Offspring remained with dams for 21 days. The control group consisted of rats that consumed water without lead. In adulthood, male offspring from both groups (lead-exposed and control) were pretreated with 7-nitroindazole (nNOS blocking agent) (10.0 mg/kg ip) or saline (1.0 ml/kg ip), 30 min before AMPH (1.0 mg/kg ip). After another 30 min, and 15 min before termination, all rats were injected with 6-$[^3H]$-D-glucose (500 Ci/kg ip). Brain specimens were taken (striatum, frontal cortex, hippocampus, and thalamus with hypothalamus, and pons with medulla oblongata) for determination of radioactivity in a liquid scintillation counter. We found that lead did not alter $[^3H]$glucose uptake in brain regions studied (with exception of frontal cortex) but that AMPH increased $[^3H]$glucose uptake in the striatum, frontal cortex and hippocampus, and that the AMPH effect was lessened in the hippocampus of lead-exposed rats. Moreover, the AMPH effect on $[^3H]$glucose uptake in the frontal cortex, hippocampus, thalamus with hypothalamus and pons of control rats was potentiated by 7-NI pretreatment. Similar effect was observed in lead-intoxicated rats (striatum, frontal cortex and hippocampus). These results indicate that NO modulates AMPH-induced $[^3H]$glucose uptake in the brain of rats prenatally exposed to lead.

Key words: lead, amphetamine, 7-nitroindazole, CNS, $[^3H]$glucose, rats

Abbreviations: AMPH – amphetamine, CNS – central nervous system, DA – dopamine, DAT – dopamine transporter, DPM – disintegration per minute, 7-NI – 7-nitroindazole, NO – nitric oxide, nNOS – nitric oxide neuronal syntase
Introduction

The neurotoxic heavy metal, lead, is a major contaminant of the environment, due to its high natural abundance, massive industrial use, and being a frequent pollutant of cheaper gasoline. The primary sources of environmental pollution, and consequently health hazards by lead, consist of leaded paints, leaded gasoline and polluted drinking water. Lead is readily transported in blood and through the blood-brain-barrier, to reach the central nervous system (CNS) and destroy nerves [1].

Clinical and experimental studies indicate that young developing organisms are more susceptible to lead neurotoxicity, and, therefore, many studies have been focused on behavioral changes, which are linked to lead consumption during developmental stages. It has been demonstrated in children that exposure to lead results in lower IQ values, attention-deficit disorder, and in learning and memory deficits [9]. Numerous studies have indicated that exposure to environmental lead adversely affects multiple neurotransmitter receptor systems, including noradrenergic, cholinergic, GABAergic and mostly dopaminergic neurons, suggesting that this harmful pollutant is heavily involved in the CNS mechanisms [11, 29].

Amphetamine (AMPH) is another psychomimetic and neurotoxin mostly for the central dopaminergic system [15, 23]. AMPH promotes dopamine (DA) release and blocks reuptake *via* its competition for DA at the DA transporter (DAT) [24, 27]. The ensuing increase in synaptic DA presumably enhances oxygen free radical formation highly neurotoxic species for dopaminergic neurons [16, 28]. Fe^{2+} plays an important catalytic role through the Fenton reaction [10]. The enhanced DA release into microdialysate of the striatum by AMPH was described [6] and confirmed in our laboratory [21]. This effect was modulated by 7-nitroindazole (7-NI), a neuronal nitric oxide (NO) synthase inhibitor (nNOS) [19, 22].

Glucose is the major energy source to the body, and especially to the CNS. Many agents influence the glucose supply and its uptake by brain. In our laboratory, we found that central DA receptor agonists such as quinpirole, 7-OH-DPAT and SKF 38393 modify[^H]-glucose uptake by brain [3, 32]. NO similarly modifies[^H]-glucose uptake in the brain [2, 16]. Because AMPH has become a common substance of abuse, there is a question as to whether AMPH might damage the central dopaminergic system, particularly where there might have been environmental exposure to lead. Therefore, the aim of this study was to examine the effect of AMPH on[^H]-glucose uptake in the brain of rats prenatally exposed to lead, and determine if NO might be involved in the process.

Materials and Methods

Pregnant Wistar rats, 200–220 g, were group-housed in a well-ventilated room, thermostated at 22 ± 2°C and under a 12 h light : 12 h dark cycle. From day “1” of pregnancy, when vaginal plugs were observed, rats were single-housed, having free access to pelleted food and water. Individual dams received either tap water with no additive (controls) or tap water containing 250 ppm lead acetate – Pb(CH₃COO)₂ · 3H₂O (POCH Ltd, Gliwice, Poland). Fluid consumption of each dam was monitored daily, and on the day of parturition the drinking water with lead was replaced by tap water. The number of pups (from control and lead-exposed dams) was adjusted (usually reduced) to six per litter (preferably males remaining); pups remained with dams until weaning on the 21st day after birth, after which all male pups from each group were pooled, and then randomly housed three per cage until the age of 2 months.

Male offspring from dams that consumed tap water (with no additive; control group) or 250 ppm lead acetate during pregnancy (5 rats per group in both cases) were pretreated with saline (1.0 ml/kg ip) or 7-NI (Sigma, St Louis, MO, USA) (10.0 mg/kg ip) and 30-min later received either saline (1.0 ml/kg ip) or AMPH (amphetamine sulfate, Sigma) (1.0 mg/kg ip). After another 30-min 6[^H]-D-glucose (Amersham Radiochemicals, Pittsburgh, PA, USA; 41 Ci/mmol) was injected ip at a dose of 0.5 μCi/g. Rats were sacrificed 15 min after administration of 6[^H]-D-glucose, for excision of the brain which was immediately placed on ice for dissection of the striatum, cerebral cortex, hippocampus, hypothalamus and pons. Each tissue sample was weighed and placed in a 20-ml scintillation vial containing 1 ml of Soluene-350 (Packard Inc., Downers Grove, IL, USA). Each vial was then tightly sealed and incubated at 37°C for 48 h, for solubilization of tissue. Scintillation cocktail (10 ml, Dimilume-30, Packard Inc., Downers Grove, Illinois, USA) was added.
IL, USA) was then added, and vials were briefly vortexed and counted in a scintillation counter (Liquid Scintillation Counter, DSA 1409, Wallac, Finland). Results are presented as DPM (disintegrations per min) per 100 mg of wet tissue (mean ± SEM) for each group.

A one- or two-way analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls was used to test the difference between groups for significance. A “p” value of 0.05 or less was used to indicate significant difference.

Experiments were accepted and approved by the Local Ethics Committee for Animal Research (permission no 20/03 issued on 27.05.2003).

Results and Discussion

[3H]Glucose uptake (DPM) was unaltered in the examined brain regions (with exception of the frontal cortex) of rats prenatally exposed to lead in comparison to control (Tab. 1). This is in accordance with our previous findings [4, 31]. 7-NI alone decreased [3H]-glucose uptake in the striatum and hippocampus of controls being without effect in other brain regions. Quite opposite effect was seen in lead-exposed rats; significant increase was observed in the striatum, frontal cortex and pons with medulla oblongata. However, AMPH (1.0 mg/kg ip) increased [3H]glucose uptake in the striatum, frontal cortex, and hippocampus of control, and the AMPH effect was lessened in the hippocampus of lead-exposed rats. Finally, the AMPH effect on [3H]glucose uptake in the frontal cortex, hippocampus thalamus with hypothalamus and pons of control rats was potentiated by 7-NI. Similar effect was observed in lead intoxicated rats (in the striatum, frontal cortex and hippocampus) (Tab. 1).

As indicated previously, glucose is the main energy source for the CNS. It should be emphasized that prenatal lead exposure induces many behavioral changes in rats challenged acutely with DA receptor agonists or antagonists [4, 5]. Lead exposure interferes with

<table>
<thead>
<tr>
<th>Part of the brain</th>
<th>Control DPM/100 mg of wet tissue</th>
<th>Prenatally exposed to lead DPM/100 mg of wet tissue</th>
<th>Two-way analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>AMPH</td>
<td>7-NI</td>
<td>7-NI + AMPH</td>
</tr>
<tr>
<td>Striatum</td>
<td>32210.2 ± 1511.0</td>
<td>49757.0 ± 1200.9</td>
<td>26503.4 ± 605.5</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>39366.4 ± 3104.1</td>
<td>53827.4 ± 1392.9</td>
<td>35722.4 ± 1408.8</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>32288.6 ± 1551.2</td>
<td>57325.0 ± 1319.1</td>
<td>49317.8 ± 1017.1</td>
</tr>
<tr>
<td>Thalamus with</td>
<td>32027.4 ± 1419.1</td>
<td>27129.2 ± 748.8</td>
<td>31072.8 ± 1222.8</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>± 3057.5 ± 1200.9</td>
<td>± 3578.7 ± 1367.1</td>
<td>± 26335.8 ± 746.0</td>
</tr>
<tr>
<td>Pons with medulla</td>
<td>29505.4 ± 763.6</td>
<td>28147.8 ± 712.3</td>
<td>33161.0 ± 1160.4</td>
</tr>
<tr>
<td>Oblongata</td>
<td>± 2803.0 ± 783.2</td>
<td>± 26462.0 ± 720.9</td>
<td>± 31048.8 ± 833.4</td>
</tr>
</tbody>
</table>

DPM – disintegrations per minute, AMPH – amphetamine 1.0 mg/kg ip, 7-NI – 7-nitroindazole 10.0 mg/kg ip, * p < 0.05 as compared to appropriate control (saline vs. AMPH; saline vs. 7-NI), † p < 0.05 as compared to amphetamine-treated group (AMPH vs. 7-NI+AMPH), ‡ p < 0.05 control compared to lead-exposed group (saline vs. saline)
central dopaminergic transmission in the brain [4, 5, 20]. AMPH, a psychostimulant with some neurotoxic potential for dopaminergic neurons, exerts stimulating effects on the central dopamine receptors, inducing many behavioral changes including stereotyped behaviors [13, 17, 26, 27]. Sokoloff et al. [30] reported that a higher dose of AMPH (5.0 mg/kg) induced an increase in 2-[14C]deoxyglucose utilization in nearly all regions of the brain. Lower AMPH doses, as in the present study, exert a much weaker effect or are without effect [12]. The presently reported results are in general agreement with related studies by others [7, 18, 25, 33], although in contrast to our study, many other researchers utilized a higher AMPH dose or multiple vs. single injection in our experiment. In our other studies, alternate central dopamine receptor agonists (quinpirole, SKF 38393, 7-OH-DPAT) were similarly found to increase [3H]glucose uptake in the brain [2, 3]. It is difficult to account for AMPH-enhanced [3H]glucose uptake in the striatum, frontal cortex and hippocampus after 7-NI. Although one must cognize that in vivo brain microdialysis studies demonstrate increased DA efflux in the striatum after AMPH (1.0 mg/kg ip), which attains a maximum response (approx. 1000%) 20–40 min after injection, and concomitant reduction in extraneuronal DOPAC and HVA levels [21]. Previously, we also demonstrated that nNOS inhibition (by 7-NI administration) potentiated AMPH-evoked DA release in the neostriatum [22]. If the DA plays some role in the AMPH-enhanced [3H]glucose uptake in the brain, it might serve some explanation of 7-NI potentiated phenomenon observed in the current study. Consequently, because lead affects NO production by interference with the enzymatic activity of nNOS [8], the effect of 7-NI on AMPH-increased glucose utilization after AMPH administration in lead intoxicated rats was much more pronounced than in control animals. In our previous studies, we showed that the non-specific NOS inhibitors L-NAME and L-NMNA, and NO donors such as arginine and molsidomine, did modify [3H]glucose uptake in the brain of intact rats [2, 16]. Notably, Kelly et al. [14] found no effect of 7-NI on glucose utilization in the brain of rats, which is only partly in accord with our results. In summary, the results demonstrate that NO modulates [3H]glucose uptake in the brain after AMPH injection of rats prenatally exposed to lead.

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References:


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