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Original Research

Developmental Lead Exposure Impairs Anxiolytic-Like Effects of Diazepam and 8-OH-DPAT in Male Wistar Rats

P. Nowak[1]*, R. M. Kostrzewa[2], B. Adamus-Sitkiewicz[1], R. Brus[1]

[1]Department of Pharmacology, Medical University of Silesia, Jordana 38, 41-808 Zabrze, Poland
[2]Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA

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Abstract

The effects of developmental lead (Pb^{2+}) exposure on the anxiolytic-like effect of diazepam (5.0 mg/kg IP) and 8-OH-DPAT (0.3 mg/kg IP) were studied. Wistar dams were exposed to 250 ppm lead acetate in drinking water during pregnancy. Control rats were derived from dams that consumed tap water, and had no exposure to Pb^{2+} afterwards. Male offspring were tested at the age of 12 weeks. We studied the anxiolytic-like effect of diazepam and 8-OH-DPAT in an elevated plus maze device and the Vogel conflict test. Diazepam in doses of 5.0 mg/kg IP significantly increased the percentage of time spent on open arms in control rats being without effect in Pb^{2+}-exposed animals. 8-OH-DPAT 0.3 mg/kg IP increased the percentage of time spent on open arms in both experimental groups (control and Pb^{2+}), but the anxiolytic-like effect was much more pronounced in Pb^{2+}-intoxicated animals. The benzodiazepine anxiolytic diazepam produced a significant effect in the Vogel conflict test in control rats. A 5.0 mg/kg dose of those drugs caused a significant increase in the number of electric shocks rats received. In the ontogenetically Pb^{2+}-exposed rats diazepam also augmented the number of shocks accepted, but this effect was much less pronounced than in control animals. Conversely, 8-OH-DPAT at doses of 0.3 mg/kg IP was without effect in both tested groups as far as the anticonflict effect is concerned. The results of the present report demonstrated that exposure to Pb^{2+} during pregnancy induced hypersensitivity to 5-HT_{1A} agonist mediated anxiolytic-like effect but attenuated that of benzodiazepine (diazepam).

Keywords: lead, anxiety, prenatal, exposure, rats

Introduction

Epidemiological and experimental studies have provided consistent evidence that lead (Pb^{2+}) is a well-known neurotoxicant and a risk factor for neurologic and psychiatric disorders in humans. Recent studies have also demonstrated that exposure to environmental Pb^{2+} essentially affects a variety of neurotransmitter systems and causes a wide range of long-lasting adverse effects, especially in developing brains [1-3].

In some previous studies we showed that prenatal Pb^{2+} exposure strongly affects dopaminergic, serotoninergic and cholinergic systems in the rat's brain. For example profound changes in neurotransmitter synthesis and metabolism in the striatum and frontal cortex were observed, also behavioral responses to specific agonist administration were vividly modified [4-12]. On the other hand, there are scarce data pointing out Pb^{2+}-induced alternation in the

e-mail: pnowak@slam.katowice.pl

level of the "basal" anxiety and hyper- or hyposensitivity to anxiolytic-like effects of benzodiazepines, ethanol and other similarly acting agents [13-15].

Conversely, it is worth knowing that anxiety-like disorders affects one-eighth of the total population world-wide and have become a very important area of research interest in psychopharmacology [16]. Benzodiazepines are still the most frequently prescribed drugs for the treatment of anxiety disorders, despite their undesirable side-effects such as memory disturbances, sedation and physical dependence [17]. Also serotonin (5-HT) is involved in a variety of brain function (e.g. thermoregulation, pain, sleep, appetite, aggression etc.), and seems to be strongly engaged in anxiety and mood regulation in mammalians [18]. Of the many 5-HT receptors discovered [19], the 5-HT_{1A} receptor has attracted attention during recent years as a potential target for anxiolytic and antidepressant drugs [20]. The 5-HT_{1A} receptor was discovered and characterized from biochemical and pharmacological studies of 8-OH-DPAT, which binds with high affinity and selectively to the 5-HT_{1A} receptor.

It was shown that reduced sensitivity to benzodiazepine and reduced $GABA_A$ receptor density have both been reported in panic disorder patients [21]. Interestingly, a lower 5-HT_{1A} receptor binding has also been found in panic disorder patients [22], implicating a connection between the 5-HT and GABA systems in the pathogenesis of anxiety.

The purpose of this work was to study whether developmental Pb^{2+} exposure (Pb-acetate, 250 ppm during pregnancy) modifies behavioral responses to diazepam (benzodiazepine receptor agonist) and 8-OH-DPAT (5-HT_{1A} receptor agonist) in male offspring rats.

Materials and Methods

Substances

The following substances were used in this study: $Pb(CH_3COO^-)_2 \cdot 3H_2O$ (POCH, Gliwice, Poland), diazepam (Jelfa, Poland), 8-OH-DPAT (R-(+)-8-hydroxy-dipropylaminotetralin HBr) (Sigma Chemicals, St. Louis, MO, USA).

Rats and Treatment

Pregnant Wistar rats each weighing 200-220 g, were used in this study. They were housed in a well-ventilated room, thermostated to 22±2ºC and under a 12 h light / 12 h inversed darkness cycle. From day "1" of their pregnancy, when vaginal plugs were found, the rats were kept one per cage with free access to pelleted food (Altromin-1324, Lage, Germany). The first group received tap water, the second group water containing 250 ppm lead acetate. On the day of parturition the lead-containing water was replaced by tap water. Control litters and Pb^{2+}- exposed litters were maintained in separate litters during postnatal development. Weaning was on the 21st day after birth. At 12 weeks of age, experimental testing was initiated. The local Bioethical Committee for Animals, at the Medical University of Silesia approved the experiment (certificate no. 20/03). All procedures, reviewed and approved by the Institutional Animal Care Committee, are in accordance with the principles and guidelines described in the NIH booklet *Care and Use of Laboratory Animals.*

The number of control and Pb^{2+}-treated rats in each experiment was 10 per tested group.

Elevated Plus Maze

Anxiety-like behavior in the elevated plus maze was measured according to Pellow et al. [23]. The apparatus was made of wood painted black with two opposing open arms (50 x 10 cm) without legs and two opposing open arms of the same size with 40 cm high walls. The arms were attached to a central square (10 cm^2) shaped as plus sign. The whole device was placed 50 cm above the floor. Rats from both tested groups were injected either with saline (1.0 ml/kg IP) or with diazepam (5.0 mg/kg IP), or 8-OH-DPAT (0.3 mg/kg IP). Immediately after injection, rats were placed back into their home cage for 60 min before the 5-min plus maze test. Each animal was placed in the central square of the plus maze, facing an enclosed arm; an arm entry was defined when all four paws entered an arm. The following scores were recorded: the number of entries into open and closed arms and time spent in both, open and enclosed arms. Scores were presented as open/total time on open arms and absolute open arms entries as anxiety indexes. The absolute number of entries into enclosed arms reflected general motor activity. The maze was cleaned after each rat was tested.

Vogel Conflict Drinking Test

A modification of the method of Vogel et al. [24] described below, was used. Male Wistar rats were water-restricted for three consecutive 24-h periods. At the end of the first and the second 24-h period, animals were placed in the test chamber containing a drinking bottle and were allowed to drink freely for 15 min. At the end of the third 24-h period, control and Pb^{2+} rats were administered with saline (1.0 ml/kg IP) or diazepam (5.0 mg/kg IP), or 8-OH-DPAT (0.3 mg/kg IP). 60 min later they were placed in the test chamber and allowed to drink for 30 s. Immediately afterwards, drinking attempts were punished with an electric shock, between the grid floor and the drinking spout (0.5 mA; 250 ms) that was triggered every 20th lick.

Data Analysis

Group differences were assessed by a two-way analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls. A P value <0.05 was taken as the level of significant difference.

Results

Elevated Plus Maze

Diazepam in a dose of 5.0 mg/kg IP significantly increased the percentage of time spent on open arms in control rats (in comparison to saline treatment) being without effect in this regard in Pb^{2+}-exposed animals. The differences between both examined groups were statistically significant (two-way analysis of variance, groups $F=0.330$ $p<0.568$; substances $F=5.315$ $p<0.027$; both factors $F=3.580$ $p<0.066$). Diazepam did not modify the number of entries into open arms in control and in Pb^{2+}-exposed rats (groups $F=0.081$ $p<0.777$; substances $F=0.009$ $p<0.929$; both factors $F=1.524$ $p<0.225$). The number of entries into closed arms were significantly decreased after diazepam administration in both experimental groups (Fig. 1) (groups $F=1.031$ $p<0.316$; substances $F=38.081$ $p<0.001$; both factors $F=0.152$ $p<0.698$).

8-OH-DPAT 0.3 mg/kg IP increased the percentage of time spent on open arms in both experimental groups (control and Pb^{2+}), but the anxiolytic-like effect was much more pronounced in Pb^{2+} intoxicated animals (two-way analysis of variance, groups $F=5.342$ $p<0.026$; substances $F=111.7$ $p<0.001$; both factors $F=2.505$ $p<0.122$). 8-OH-DPAT in a similar way enhanced the number of entries into open arms in both tested groups (groups $F=0.272$ $p<0.605$; substances $F=25.088$ $p<0.001$; both factors $F=0.043$ $p<0.835$). In addition, 8-OH-DPAT did not influence the number of entries to the closed arms, suggesting that the behavioral effect observed in control and Pb^{2+}-exposed rats was not due to an increase in exploratory activity (Fig. 2) (groups $F=0.008$ $p<0.929$; substances $F=0.200$ $p<0.657$; both factors $F=2.893$ $p<0.097$).

Vogel Conflict Drinking Test

The benzodiazepine anxiolytic diazepam produced a significant effect in the Vogel conflict test in control rats *vs* saline. A 5.0 mg/kg dose of diazepam caused a significant increase in the number of electric shocks rats received (i.e., anticonflict effect) (4.11 ±1.33 v. 20.2 ±3.5). In the ontogenetically Pb^{2+}-exposed rats diazepam also significantly increased the number of shocks accepted; 4.44 ±1.77 (saline) *vs* 11.70 ±1.72 (diazepam), respectively, but this effect was much less pronounced than in control animals (two-way analysis of variance, groups $F=3.176$ $p<0.083$; substances $F=26.048$ $p<0.001$; both factors $F=3.735$ $p<0.061$). Conversely, 8-OH-DPAT at a dose of 0.3 mg/kg ip did not evoke significant effect both in control and Pb^{2+}-exposed rats as far as the anticonflict effect is concerned (Fig. 3) (groups $F=0.049$ $p<0.825$; substances $F=3.318$ $p<0.076$; both factors $F=1.044$ $p<0.313$).

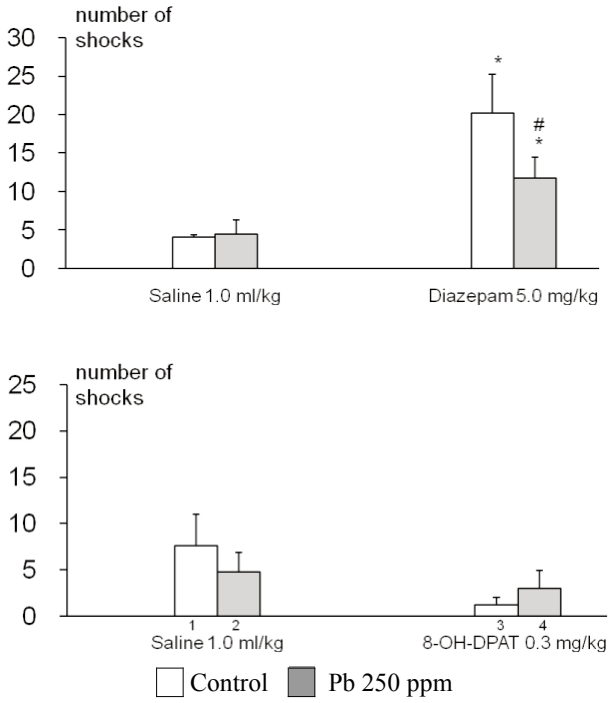

Fig. 1. Effect of ontogenetic Pb^{2+} exposure on the number of shocks rats received after saline (1.0 ml/kg IP), diazepam (5.0 mg/kg IP) or 8-OH-DPAT (0.3 mg/kg IP) treatment of adult rats (n=10).
* p<0.05; Control (1) vs. Control + Diazepam (3); Pb 250 ppm (2) *vs* Pb250 ppm + Diazepam (4)
# p<0.05; Control+Diazepam (2) *vs*. Pb 250 ppm + Diazepam (4)

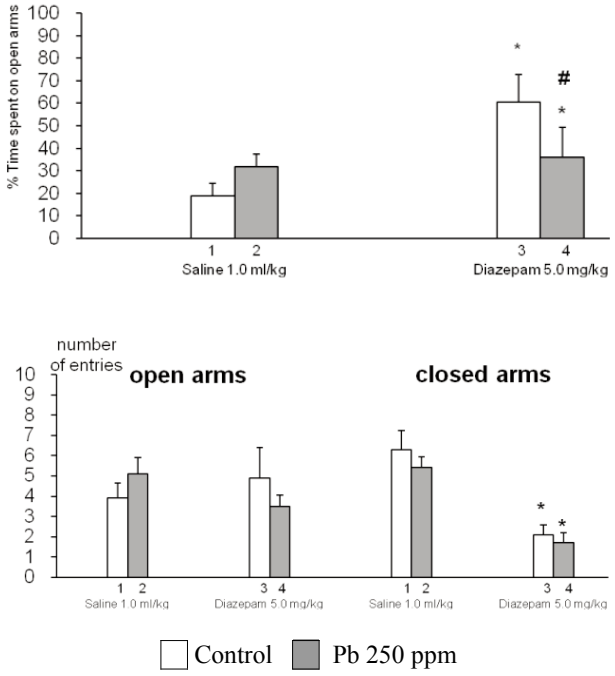

Fig. 2. Effect of ontogenetic Pb^{2+} exposure on percentage of time spent on open arms and the number of entries into open and closed arms of elevated plus maze after saline (1.0 ml/kg IP) or diazepam (5.0 mg/kg IP) treatment of adult rats (n=10).
* p<0.05; Control (1) vs. Control + Diazepam (3); Pb 250 ppm (2) *vs* Pb250 ppm + Diazepam (4)
# p<0.05; Control+Diazepam (2) *vs*. Pb 250 ppm + Diazepam (4)

Discussion

The main finding of the present study is that Pb^{2+} exposure of mothers during gestation adversely affects susceptibility to anxiolytic-like effects of diazepam (benzodiazepine) and 8-OH-DPAT (5-HT_{1A} agonist) in offspring rats. Based on literature data diazepam expressis anxiolytic-like effect in rats and mice in doses ranging from 2.5–10 mg/kg [27, 28] and 8-OH-DPAT from 0.01-0.5 mg/kg [29, 30]. With the above as a background, in the present study we chose to test the intermediate doses of each substance; 5.0 mg/kg for diazepam and 0.3 mg/kg for 8-OH-DPAT, respectively.

In the current study we demonstrated the lack of Pb^{2+}-exposure effect on basal anxiety (after saline administration) evaluated in the elevated plus maze and Vogel drinking test (Figs. 1-3). This is in line with Moreira et al. [14], but others showed different results [15]. Diazepam administered 30 min before testing in a dose of 5.0 mg/kg IP significantly increased the percentage of time spent on open arms in control rats (in comparison to saline treatment), being in this regard without effect in Pb^{2+}-exposed animals. It must be added that at the same time diazepam suppressed locomotor activity in both tested groups; accordingly, a decrease in the number of entries into closed arms was noted (Fig. 2). Despite that action, an anxiolytic-like effect was still observed in control animals. The above may indicate on diminished sensitivity to diazepam anxiolytic-like effect in Pb^{2+} treated rats. Also, the number of shocks accepted in Vogel conflict test was lower in Pb^{2+} group than in control after diazepam administration. The results of the present study contrast with Virgolini et al. [13] who found that rats exposed to Pb^{2+} (220 ppm) during ontogeny and lactation were hypersensitive to the anxiolytic-like effect of ethanol (2.0 g/kg) and showed greater voluntary intake of this drug. Nevertheless, one must be cognizant that ethanol is a very unspecific drug which acts through the variety of neurotransmitter systems. Also, differences in strain, age, the procedure of Pb^{2+} exposure and time testing make comparisons difficult. It must be added that there are no other reports examining this issue (benzodiazepine action in Pb^{2+} treated rats).

In the present experiment we also showed that 8-OH-DPAT 0.3 mg/kg IP increased the percentage of time spent on open arms in both experimental groups (control and Pb^{2+}), but the anxiolytic-like effect was much more pronounced in Pb^{2+} intoxicated animals ($p<0.05$) (Fig. 3). 8-OH-DPAT also enhanced the number of entries into open arms (regarded as the second index of anxiety-like behavior) in both tested groups. In addition, 8-OH-DPAT did not influence the number of entries to the closed arms, suggesting that the behavioral effect observed in control and Pb^{2+}-exposed rats was not due to an increase in exploratory activity. To summarize, prenatal Pb^{2+} exposure enhanced anxiolytic-like effects mediated by 5-HT_{1A} receptor agonist.

Surprisingly, in the Vogel drinking test 8-OH-DPAT administered in a dose of 0.3 mg/kg IP was without effect, which is difficult to explain. 5-HT_{1A} receptors are located presynaptically on the soma and dendrites of the serotonergic neurons of the midbrain raphe nuclei and act as inhibitory autoreceptors, and postsynaptically to the serotonergic neurons in the forebrain areas [25]. The respective roles of (pre- and postsynaptic) 5-HT_{1A} receptors in the control of behavior in the Vogel conflict test and other paradigms is a complex question to which the answer depends upon the model (type of anxiety), serotoninergic tone, gender, the level of stress of the drug under study as well as its dose and site of administration. Perhaps the dose of 8-OH-DPAT used in the elevated plus maze in the present paper was adequate for testing but too high to "properly" respond to a conflict situation (Vogel conflict) [26].

The precise molecular mechanism of the observed abnormalities in Pb^{2+}-exposed rats remains very complex. Xiao et al. [31] provided electrophysiological evidence (from developing hippocampal slices) that Pb^{2+} inhibited action potential-dependent GABA release by inhibiting presynaptic voltage-gated calcium channels. Also Braga et al. [32] showed that Pb^{2+} increases the frequency of GABA- and glutamate-mediated miniature postsynaptic currents (MPSCs) recorded by means of the patch-clamp technique from cultured hippocampal neurons. Given that synaptic activity is a key mechanism for the establishment of stable

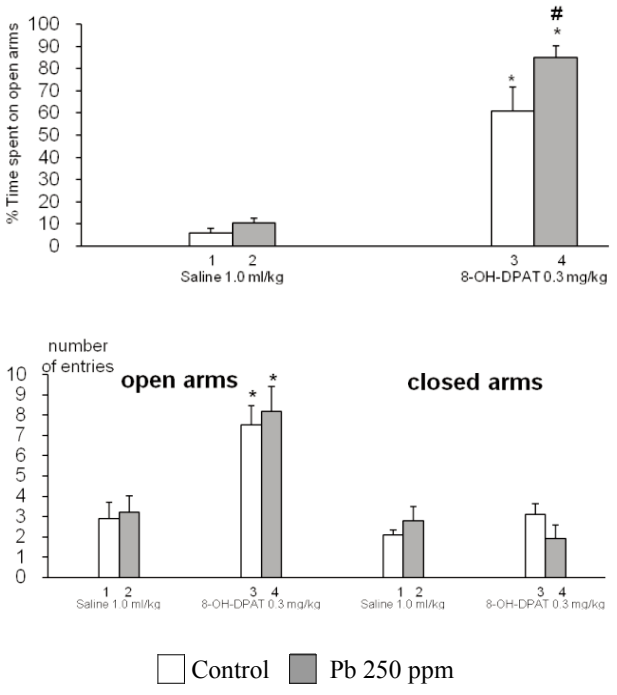

Fig. 3. Effect of ontogenetic Pb^{2+} exposure on percentage of time spent on open arms and the number of entries into open and closed arms of elevated plus maze after saline (1.0 ml/kg IP) or 8-OH-DPAT (0.3 mg/kg IP) treatment of adult rats (n=10).

* $p<0.05$; Control (1) *vs*. Control + 8-OH-DPAT (3); Pb 250 ppm (2) vs Pb250 ppm + 8-OH-DPAT (4)

# $p<0.05$; Control + 8-OH-DPAT (2) *vs*. Pb 250 ppm + 8-OH-DPAT (4)

synaptic connections early in the development, it is possible that, by interfering with spontaneous transmitter release, Pb^{2+} has lasting effects on neuronal maturation and plasticity. Since GABA-ergic and serotoninergic neurotransmissions are particularly involved in the central nervous system anxiety effects of diazepam and 8-OH-DPAT, behavioral effect to low doses of Pb^{2+} during gestation could be related to changes in the development of these neuronal systems.

In summary, this is the first report demonstrating that exposure to Pb^{2+} during pregnancy induces hypersensitivity to 5-HT_{1A} agonist mediated anxiolytic-like effect but attenuates that of benzodiazepine (diazepam). As has been noted, such small doses of Pb^{2+} given during the prenatal period has a powerful effect on the regulation of anxiety in offspring rats.

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