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Effect of pizotifen on convulsive effect of *meso*-tetra-4*N*-methyl-pirydyl-porphyrin (P) in mice

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Abstract: In this report we present effect of pizotifen, an antagonist of serotonin (5-hydroxytryptamine; 5-HT) receptors, on P-induced convulsions in mice. Experiments were conducted on male mice Balby. Convulsive effect P was determined using the following measures: percentage of mice with seizures, the number of seizure episodes/2 h, the latency time of the beginning P-induced seizure activity in mice and P-induced seizure activity of mice determined by score of Racine. Pretreatment mice with pizotifen (0.5 mg/kg ip) did not prevent convulsive effect P, applied ip at the dose of 50 μmol/kg ip (59.3 mg/kg) but significantly shortened the latency time of the beginning of P-induced seizure activity of mice. We conclude that central serotonin receptors are involved in the mechanism of P-induced convulsions.

Keywords: *meso*-tetra-4*N*-methylpirydyl-porphyrin, convulsive effect, mice, pizotifen

INTRODUCTION

Porphyrins, a class of compounds playing a key role in metabolism of living organisms, may also exert toxic effects. Porphyrias, a group of disorders, caused in humans by inherited or rarely acquired deficiency of one of eight enzymes of

the pathway of heme synthesis and accumulation of one of porphyrins are result of their toxicity [1-4]. Among several symptoms of porphyrias are neurological manifestations, such as seizures, paresis of the upper and lower extremities and different psychiatric disorders [3, 4].

Porphyrins are being in use in photodynamic tumor therapy. Practically used photosensitizer Fotophrin II is a mixture of hematoporphyrins, hydroxyethinwinyldeuteroporphyrin and protoporphyrin [5, 6]. Cathionic water-soluble metalloporphyrins were used for reductive dechlorination of carbon tetrachlorides polluting the soil [7].

Higher doses of porphyrins may also induce convulsions in experimental animals [8-11]. Noske et al. reported that intracerebral administration of hematoporphyrin derivatives induced local damage of brain tissue [12]. In our previous reports convulsive effect of synthetic porphyrin *meso*-tetra-4*N*-methylpirydyl-porphyrin (P) in rats and mice was demonstrated [8-11,13]. It was found that injection P into the lateral brain ventricle (icv) induced seizures in rats. This effect was blocked by prior ip administration of diazepam but at high dose of 40 mg kg^{-1} [11]. Moreover it was demonstrated that β-adrenergic receptors are involved in the mechanism of P-induced convulsive effect in mice as prior administration of propranolol (1 mg kg^{-1} ip), β-adrenergic antagonist, prevented the convulsive effect of ip injected P at the dose of 50 μmol kg^{-1} in mice [13]. On the other hand α-adrenergic antagonist dibenzyline did not inhibit convulsive effect P [13]. In this report we present effect of pizotifen, an antagonist of serotonin (5-hydroxytryptamine) receptors, on P-induced convulsions in mice.

MATERIALS AND METHODS

Experiments were conducted on male Balby mice of 20-35g body weight obtained from the Animal Farm of the Medical University of Silesia in Katowice, Poland. Animals were housed in a room at 22 ±2 °C on a 12:12 light:dark cycle (lights on at 06:00 am) with free access to standard food and water.

On the day of experiment *meso*-tetra-4*N*-methylporphyrin (P), or pizotifen were injected intraperitoneally (ip), and the animals were observed for the next 2 hours. The percentage of animals that manifested convulsive effects for each experimental and control group, the number of seizure episodes, and the latency time for the beginning of convulsive effect were recorded. Seizure activity was evaluated using the score of Racine [14].

Results obtained for the percentage of animals that manifested convulsive effect were analyzed using a chi-square test [15], while results obtained from

other tests were subjected to analysis of variance (ANOVA) and the post-ANOVA Dunnett's test [15].

The experimental protocol was approved by the local ethic committee of the Medical University of Silesia in Katowice (NN-013-387/99).

RESULTS AND DISCUSSION

Intraperitoneal (ip) injection P at the dose of 50 μmol kg^{-1} (59.3 mg kg^{-1}) exerts convulsive effect in mice as 68% of animals appeared convulsions (Figure 1), the mean number of seizure episodes in mice was 3.8/2h (Figure 3) and the seizure activity of mice determined by score of Racine was 1.6 (Figure 4) while the latency time of the beginning of P-induced seizure activity in mice was shortened to 5000 s (Figure 2). These results confirmed our previous finding that P injected ip at the dose of 50 μmol kg^{-1} (59.3 mg kg^{-1}) exerts convulsive effect in mice [9]. Such dose P of 50 μmol kg^{-1} (59.3 mg kg^{-1}) was chosen for evaluation effect of pizotifen on P-induced convulsive effect in mice, as in our previous paper was evaluated effect of α-and β-adrenergic antagonist on convulsive effect P applied at the same dose of 50 μmol kg^{-1} ip in mice [13]. Moreover it was previously demonstrated that convulsive effect P in mice was a dose-dependend as 2-fold higher dose P of 100 μmol kg^{-1} induced much stronger convulsive effect [13]. The present study displayed that pizotifen an unselective antagonist of central postsynaptic serotonin receptors [16, 17] also may block P-induced convulsive effect in mice. Pretreatment mice with pizotifen evidently decreased the number of P-induced seizure episodes (Figure 3) and the seizure activity determined by the score of Racine (Figure 4). However the percentage of animals with seizures was not decreased (Figure 1), and the latency time of the beginning of P-induced seizure activity in mice was significantly shortened (Figure 2). Thus in spite that not uniform inhibitory effect of pizotifen on P-induced convulsive effect in mice was recorded, we regard that central serotonergic receptors are involved in this P-induced effect. It was reported in several papers that serotonin receptors modulate seizures of different origin [19-21]. Administration of serotonin or its precursor 5-hydroxytryptophan increased the threshold for evoked seizures and inhibited focal and generalized seizures [20]. It was demonstrated that agonists or antagonists of different subtypes of central serotonergic receptors play important role in mechanism of seizure generation [21-23]. Moreover it was reported that propranolol, β-adrenergic antagonist significantly blocked convulsive effect P in mice [9]. Taking into account our previous reports [5, 9, 18] and the results of present study we suggest conclusion that toxic doses of porphyrins may inapair

function of different neurotransmitter systems and in this way enable a prominent enhancement activity of brain neurons manifested as seizure activity.

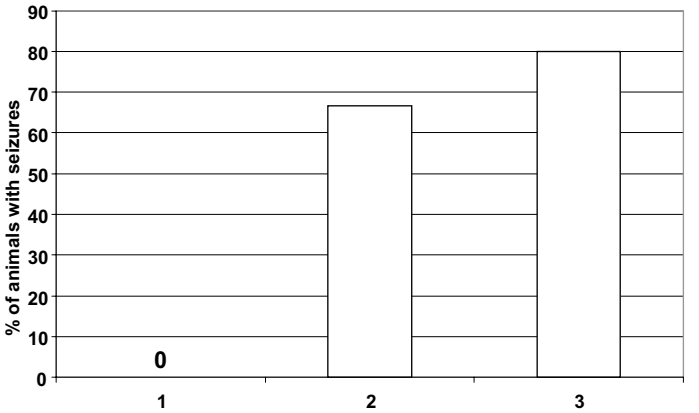

Figure 1. Pizotifen effect on percentage of mice with P-induced seizure activity.

1 - Control 0.9% NaCl 1 ml/kg ip; (n=5).
2 - P 59.3 mg/kg (50 μmol/kg) ip; (n=6).
3 - Pizotifen 0.5 mg/kg ip + after 30 min P 59.3 mg/kg (50 μmol/kg) ip; (n=5).

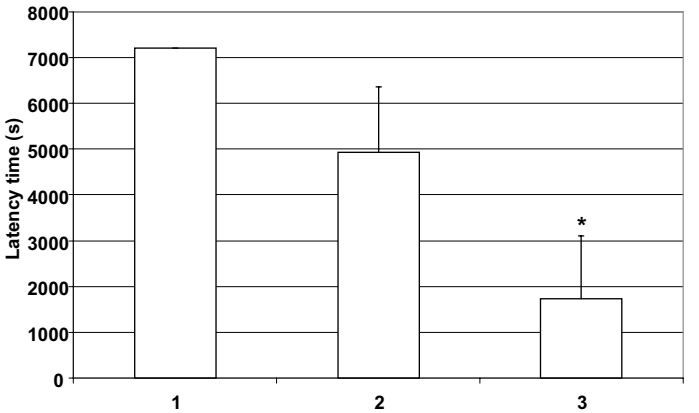

Figure 2. Pizotifen effect on the latency time of the beginning P-induced seizure activity in mice.

1 - Control 0.9% NaCl 1 ml/kg ip; (n=5).
2 - P 59.3 mg/kg (50 μmol/kg) ip; (n=6).
3 - Pizotifen 0.5 mg/kg ip + after 30 min P 59.3 mg/kg (50 μmol/kg) ip; (n=5).
(*) significance v. control (1) Dunnett's test $p<0.05$.

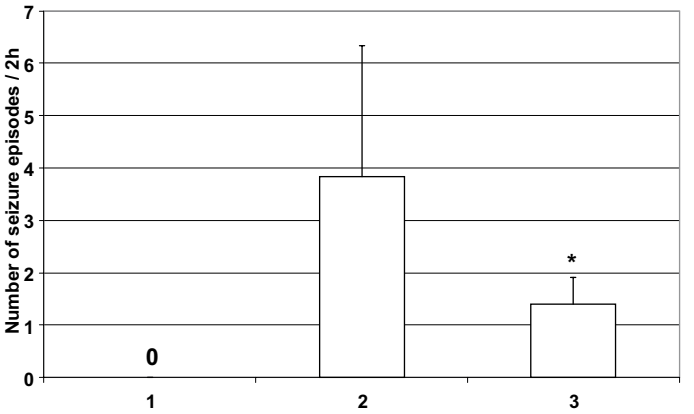

Figure 3. Pizotifen effect on the number of P-induced seizure episodes in mice.

1 - Control 0.9% NaCl 1 ml/kg ip; (n=5).
2 - P 59.3 mg/kg (50 μmol/kg) ip; (n=6).
3 - Pizotifen 0.5 mg/kg ip + after 30 min P 59.3 mg/kg (50 μmol/kg) ip; (n=5).
(*) significance v. control (1) Dunnett's test $p<0.05$.

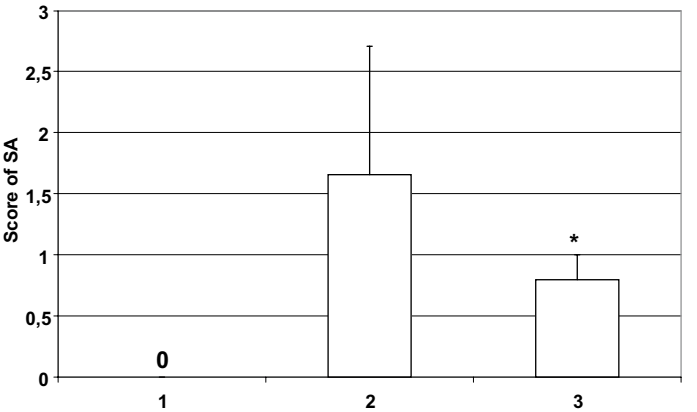

Figure 4. Pizotifen effect on the seizure activity (SA) determined by score of Racine in mice with P-induced seizures.

1 - Control 0.9% NaCl 1 ml/kg ip; (n=5).
2 - P 59.3 mg/kg (50 μmol/kg) ip; (n=6).
3 - Pizotifen 0.5 mg/kg ip + after 30 min P 59.3 mg/kg (50 μmol/kg) ip; (n=5).
(*) significance v. control (1) Dunnett's test $p<0.05$.

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