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## SHORT COMMUNICATION

# NICOTINE DIMINISHES ANTICONVULSANT ACTIVITY OF ANTIEPILEPTIC DRUGS IN MICE

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Nicotine administered acutely at subconvulsive dose of 4 mg/kg, significantly decreased the protective activity of valproate, carbamazepine, diphenylhydantoin, phenobarbital, topiramate and lamotrigine against maximal electroshock-induced tonic convulsions in mice. The obtained data may suggest that interaction between nicotine and antiepileptic drugs should be carefully considered as a cause of the therapeutic failure in epileptic patients.

***Key words:*** *nicotine, antiepileptic drugs, seizures*

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*Abbreviations:* AED – *antiepileptic drug,* CBZ – *carbamazepine,* $CS_{50}$ – *current strength in mA necessary to produce tonic hindlimb extension in 50% of the tested animals,* DPH – *diphenylhydantoin,* $ED_{50}$ – *50% effective anticonvulsant dose,* LTG – *lamotrigine,* MES – *maximal electroshock,* PB – *phenobarbital,* TPM – *topiramate,* VPA – *valproate*

## INTRODUCTION

Nicotine is one of the most heavily used addictive drugs. Cigarette smoking has been the most popular method of taking nicotine since the beginning of the 20th century. Nicotine, the major pharmacologically active component of tobacco smoke, is generally regarded to be a primary risk factor in the development of cardiovascular disorders, pulmonary disease and lung cancer [3, 7]. On the other hand, it produces many effects on the CNS, some of which may be considered to be beneficial, e.g. mood elevation, arousal, and learning and memory enhancement. The use of nicotine as a potential drug for the remission of ulcerative colitis, Alzheimer's disease and Parkinson's disease have been investigated [13, 19, 21, 22]. The behavioral effects of nicotine are attributed to an action at nicotinic receptors. The overstimulation of nicotinic receptors in the brain results in clonic-tonic convulsions, which can be suppressed by nicotine receptor antagonist, mecamylamine [4]. Recent data show a relationship between mutant nicotine acetylcholine receptors and nocturnal frontal lobe epilepsy, which may be a result of increased sensitivity of these receptors to acetylcholine [17].

To the best of our knowledge, the influence of systemic administration of nicotine upon protective activity of antiepileptic drugs (AED) has never been studied, therefore, the aim of this study was to evaluate the influence of nicotine upon protective activity of standard and new AEDs.

## MATERIALS and METHODS

The experiments, after acceptance by Local Bioethics Committee, were conducted on adult male Swiss mice weighing 20–24 g. Each experimental group consisted of 8–10 animals.

The following substances were used: nicotine [(–)-nicotine di-d-tartrate; RBI, Natick, MA, USA], valproate magnesium (VPA; Polfa, Rzeszów, Poland), diphenylhydantoin (DPH; Sigma, St. Louis, USA), carbamazepine (CBZ; Polfa, Starogard Gdański, Poland), phenobarbital sodium (PB; Polfa, Kraków, Poland), lamotrigine (LTG; Glaxo Wellcome, Kent, UK), and topiramate (TPM; Janssen Pharmaceutica, Beerse, Belgium). Nicotine and VPA were dissolved in sterile saline. CBZ, PB, DPH, TPM, and LTG were suspended in a 1% solution of Tween 80 (Loba-Chemie, Austria). All drugs were administered intraperitoneally (*ip*) in a volume of 0.1 ml/10 g at times of their peak activity.

In order to estimate the anticonvulsant $ED_{50}$ values (50% effective anticonvulsant doses), mice were pretreated with different doses of an AED and then challenged with maximal electroshock (MES, 25 mA, 50 Hz, 0.2 s) delivered by a Hugo Sachs generator with the use of ear-clip electrodes. Nicotine (4 mg/kg *ip*) was administered 5 min before electroshock. $ED_{50}$ values with 95% confidence limits were calculated. In order to evaluate the respective $ED_{50}$ values, at least four groups of mice, after receiving progressive doses of the AED, were challenged with MES. A dose-effect curve was subsequently constructed on the basis of the percentage of animals protected against the convulsions. The convulsive threshold was evaluated as $CS_{50}$, which is a current strength (in mA) necessary to produce tonic hindlimb extension in 50% of the tested animals. In order to estimate the threshold, at least four groups of mice (8–10 animals per group) were challenged with electroshocks of various intensities. Subsequently, an intensity-response curve was calculated on the basis of the percentage of mice convulsing. $CS_{50}$ values with respective 95% confidence limits were calculated. $ED_{50}$ and $CS_{50}$ values were calculated according to Litchfield and Wilcoxon [14].

## RESULTS

Nicotine (5 and 6 mk/kg) administered *ip* 5 min before electroshock lowered the convulsive threshold in a dose-dependent manner, whilst it had no effect on the convulsive threshold at the dose of 4 mg/kg (Tab. 1). Nicotine administered at subthreshold dose of 4 mg/kg *ip* significantly decreased the protective activity of VPA, CBZ, DPH, PB, TPM and LTG against MES-induced tonic convulsions in mice (Tab. 2).

*Table 1.* Effect of systemic nicotine administration upon electrical seizure threshold in mice

| Treatment | $CS_{50}$ with 95% confidence limits (mA) | p |
|---|---|---|
| Vehicle | 8.3 (7.9–8.8) | – |
| Nicotine 4 mg/kg | 8.5 (7.6–9.4) | NS |
| Nicotine 5 mg/kg | 7.6 (7.1–8.1) | < 0.05 |
| Nicotine 6 mg/kg | 6.8 (6.4–7.1) | < 0.01 |

Nicotine was administered *ip* 5 min before the test. The $CS_{50}$ value (with 95% confidence limits), which is a current strength necessary to produce convulsions in 50% of the tested animals was calculated by fitting the data by computerized probit analysis based on the method of Litchfield and Wilcoxon [14]; p value at least < 0.05 was considered statistically significant. NS – not significant

*Table 2.* Effect of nicotine upon the protective efficacy of antiepileptic drugs against maximal electroshock-induced convulsions in mice

| Treatment | Anticonvulsant drug $ED_{50}$ with 95% confidence limits (mg/kg) | p |
|---|---|---|
| VPA | 204.8 (184.0–227.8) | |
| Nicotine 4 mg/kg + VPA | 291.7 (267.5–318.0) | < 0.01 |
| CBZ | 11.1 (8.8–14.2) | |
| Nicotine 4 mg/kg + CBZ | 18.0 (15.3–21.3) | < 0.01 |
| DPH | 8.5 (6.7–10.8) | |
| Nicotine 4 mg/kg + DPH | 13.2 (11.3–15.4) | < 0.01 |
| PB | 14.6 (12.8–16.5) | |
| Nicotine 4 mg/kg + PB | 26.9 (21.4–33.8) | < 0.01 |
| TPM | 28.9 (25.2–33.2) | |
| Nicotine 4 mg/kg + TPM | 50.9 (45.4–57.1) | < 0.01 |
| LTG | 5.9 (4.5–7.8) | |
| Nicotine 4 mg/kg + LTG | 11.0 (9.9–12.1) | < 0.01 |

Nicotine was administered at the subconvulsive dose of 4 mg/kg *ip* 5 min before electroshock [9]. The $ED_{50}$ value (with 95% confidence limits), which is 50% effective anticonvulsant dose was calculated by fitting the data by computerized probit analysis based on the method of Litchfield and Wilcoxon [14]; p value at least < 0.05 was considered statistically significant. CBZ – carbamazepine; DPH – diphenylhydantoin; LTG – lamotrigine; PB – phenobarbital; TPM – topiramate; VPA – valproate

## DISCUSSION

It is widely accepted that high doses of nicotine produce clonic-tonic convulsions in animals after systemic or intracerebral administration [4, 9, 15]. Here, we report that subconvulsive doses of nicotine slightly reduced seizure threshold for electroconvulsions in mice. Moreover, low dose of nicotine, which did not affect threshold for electrically induced tonic seizures, was found to dramatically decrease anticonvulsive activity of all studied antiepileptics, VAL, CBZ, DPH, PB, LTG and TPM. Similarly, the potency of N-methyl-D-aspartate antagonists, which are known to possess anticonvulsive properties in MES test was decreased by administration of a subconvulsant dose of nicotine [15].

The nicotine-induced convulsions are centrally mediated and can be suppressed by antagonists of nicotine receptors [4]. These data argue for the involvement of nicotine receptors in producing nicotine-induced seizure activity. However, anticholinergic drugs are not included in antiepileptic armamentarium.

Recently, the genetically transmissible epilepsy, ADNFLE, has been associated with specific mutations in the gene coding for the alpha4 or beta2 subunits of nicotine receptors, which leads to altered receptor properties [8, 17]. In accordance with these results, studies performed on a strain of exon replacement mice ("L9'S knock-in"), whose alpha4 nicotinic receptor subunits have a leucine to serine mutation in the M2 region, 9' position rendering alpha4-containing receptors hypersensitive to agonists showed that nicotine induced seizures at concentrations approximately eight times lower in L9'S than in wild-type littermates [5]. Dysfunction of alpha5 or alpha7 nicotinic acetylcholine receptors has also been implicated in control of susceptibility to nicotine-induced seizures [2, 6, 21].

Nicotine is one of the most heavily used addictive drugs. Nicotine is easily absorbed through the skin and mucosal lining of the mouth and nose or by inhalation in the lungs. The amount of nicotine absorbed from an average cigarette is about 1.5 mg and plasma nicotine concentration reaches 130–200 nmol/l. Cigarette smoking results in rapid distribution of nicotine throughout the body, reaching the brain within few seconds of inhalation.

According to Kyngas [12], among the Finnish population of adults with epilepsy 28% smoked regularly, 22% occasionally and 50% never. It should be also accentuated that due to the environmental exposure, nicotine or its metabolite cotinine, which is a biomarker for tobacco smoke, were found in urine and hair of non-smokers [16, 18]. Moreover, the endogenous origin of nicotine in human was suggested, and trace amounts of nicotine were

found in serum of patients with epilepsy [1, 20]. Thus, it seems that the effect of low dose of nicotine upon the anticonvulsant action of AEDs should be carefully considered.

More than 30% of patients suffering from epilepsy have inadequate control of seizures with drug therapy [10]. The mechanism of drug resistance remains unknown. In 1995, Tischler et al. [23] proposed that P-glycoprotein, a membrane transporter, could be a cause of medically refractory epilepsy. More recently, Kwan and Brodie [11] presented the concept of refractory epilepsy as a condition characterized by progressive neuronal, cognitive and psychosocial deterioration.

Based on our results, we hypothesize that endogenous or environmental agent, nicotine, should be carefully considered as an important factor leading to the lack of the satisfactory therapeutic success in epileptic patients.

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