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## EFFECT OF VENLAFAXINE AND NICOTINE ON THE LEVEL OF NEUROTRANSMITTERS AND THEIR METABOLITES IN RAT BRAINS

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Nicotine (NIC) and venlafaxine (VEN) have been proved to exert antidepressant activity in both human and animals. The effect of antidepressant doses of NIC and VEN (our previous results) on noradrenergic (NA), dopaminergic (DA), serotonergic (5-HT) neurotransmitters and their metabolites: DOPAC, HVA and 5-HIAA in rats' hippocampus in freely moving rats were determined by microdialysis technique and HPLC method. Both drugs release 5-HT and NA, but VEN to a greater degree. DA level was affected only by VEN, however NIC extended the response of the DA system on VEN's effect. Combined administration of drugs caused the greatest changes in the 5-HT system. Both drugs contributed to reduction in neurotransmitter biotransformation.

**Key words:** *venlafaxine, nicotine, neurotransmitters, microdialysis, noradrenaline, dopamine, hippocampus*

### INTRODUCTION

The monoaminergic theory on etiology of the depressive disorder states that the underlying cause of the disease is the insufficient noradrenergic (NA) and serotonergic (5-HT) transmission in the central nervous system (CNS) (1-4) and it takes several weeks for the therapeutic effect to become evident. VEN is also known to show antidepressant effect already upon single administration (5, 6), its mechanism of action is related to inhibition of serotonin and noradrenaline reuptake in the CNS.

Increased availability of neurotransmitters in the brain caused by their increased release is characteristic of the NIC's mechanism of action. Our earlier studies (7) have shown NIC to enhance VEN's antidepressant and procognitive effect while MEC (NIC receptor antagonist) diminished these effects (8, 9). NIC is the agonist of cholinergic nicotinic receptors with proven antidepressant efficacy both in animals and humans (10-13). Our previous studies have shown pure NIC and VEN to have antidepressant as early as upon single administration (5, 7), while combined administration of NIC and VEN enhanced the antidepressant effect observed upon administration of VEN alone in animals (7).

Recent studies suggest that also other mechanisms may be responsible for antidepressant effect of drugs, for instance by increase of synaptic plasticity in the hippocampal region or by interactions between neurotransmitters in the central nervous system (1-4, 14, 15).

The undertaken study aimed at analyzing the effects of VEN and NIC on the baseline NA, DA, 5-HT and their metabolites (DOPAC, HVA and 5-HIAA) efflux in the hippocampus of rats.

### MATERIAL AND METHODS

#### *Animals*

The experiment on animals was performed in accordance with the Ministry of High Education Report of 1959, as well as UNESCO Declaration of Animals' Rights of 1978 (Paris).

Female Wistar rats (180-200 g) bought from a licensed breeder (licence of the Ministry of Agriculture; Warsaw, Poland) were used in this study. The animals were housed in standard laboratory conditions under a 12-hour light/dark cycle, light on at 6 a.m., in a temperature-controlled room at 21±2°C, humidity 60%, with free access to granulated standard food and tap water. The rats were kept four per cage (30x30x20 cm). Each experimental and control group consisted of 8 animals (the total number of animals was 32).

The control groups were given oral carboxymethylcellulose solution (0.5% CMC). The study groups were given VEN 20 mg/kg i.p. and NIC 0.2 mg/kg s.c. Animals were selected to particular groups on random basis.

The study protocol was approved by the Ethics Commission for Research on Humans and Animals at the University of Medical Sciences in Poznan.

#### *Drugs*

VEN (CAS: 93413-69-5) was from Wyeth-Ayerts Laboratories Princeton NJ (USA), (-) nicotine hydrogen tartrate salt (CAS: 54-11-5) was from Sigma-Aldrich (USA) and carboxymethylcellulose sodium salt (CMC) pure B.P.C. was from Koch-Light Laboratories Ltd. (London, England).

VEN (20 mg/kg) was suspended in the solution containing CMC and administered i.p. 30 min before the microdialysis. NIC 0.2 mg/kg was dissolved in saline and administered (s.c.) at a single dose 20 min before the microdialysis. Drug doses and administration routes were selected based on our previous studies in which NIC and VEN have shown antidepressant effect except that VEN was administered i.p. and not p.o. due to the dialysis cannula previously implanted into animals.

*Test of neurotransmitter and their metabolites release in the hippocampus of rats in vivo*

*1. Microdialysis in freely moving rats*

Release of NA, DA, 5-HT and metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC), homovaniline acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA) was measured by microdialysis in freely moving rats. To implant a vertical dialysis cannula, rats under general anaesthesia induced with administration of ketamine (75 mg/kg i.m.) and xylazine (10 mg/kg i.m.), were immobilised on a stereotaxic table (David Kopf Instrument, Tujunga, USA). Following skin incision, a hole was made in the skull through which dialysis cannula was inserted into the hippocampus according to the parameters in the rat brain atlas (16): distance B= -5.6 mm, from L= +4.8 mm and from dura mater V= -8.0 mm. Then the hole was secured with surgical wax and cannula was attached to the cranial bones using tooth cement. Following 24 hours of the surgery, cannulae in freely moving rats were connected to an injector perfusion pump and rinsed with an artificial cerebral fluid containing: 2.7 mM KCl, 1.2 mM CaCl<sub>2</sub>, 145 mM NaCl, 1.0 mM MgCl<sub>2</sub>, pH 7.4 for 2 hours with the rate of 1.5 µL/min., until a constant baseline level of the tested neurotransmitters in the extracellular space was achieved. Then, every 20 minutes 3-5 fractions would be collected to determine spontaneous release, and then the animals would receive injections of the tested drugs. Subsequent fractions of dialysates would be collected for 3 hours. Analysis of samples following microdialysis was performed using HPLC method with electrochemical detection (17).

*2. Analysis of the tested substances*

Measurement of DA, 5-HT, DOPAC, HVA and 5-HIAA, NA in hippocampus dialysates was performed using an HPLC system comprising an LC-10 AD pump (Shimadzu, Europa GmbH, Warszawa, Polska), LC-4B amperometric detector with a flow cell (BAS, IN, USA) and a BDS-Hypersil C18 analytical column (3x100 mm, 3 µm, Thermo Electron Corp., UK). Mobile phase containing: 0.1 M of monochloroacetic acid (pH=3.7 adjusted with 3 M NaOH), 0.5 mM EDTA, 25 mg/L 1-octansulphonic acid sodium salt, 5.7% methyl alcohol and 2.5% acetonitrile, was pumped at the rate of 0.5 mL/min, and the working electrode potential was +0.6V at the detection sensitivity of 2 nA/V. NA was measured using an HPLC system comprising a P580 pump (Dione, USA), a BDS-Hypersil C18 analytical column (2.0x100 mm, 3 µm, Thermo Electron Corp., UK) and an LC-4B amperometric detector with a flow cell (BAS, UniJet). NA analysis was performed at the potential of +0.6V and sensitivity of 2 nA/V. Mobile phase containing: 0.05 M phosphate buffer solution (adjusted to pH=3.7 with orthophosphoric acid), 0.5 mM EDTA, 150 mg/L 1-octansulphonic acid sodium salt, 10 mM NaCl and 1.2% acetonitrile, was pumped by the analytical column at the rate of 0.18 L/min. Chromatographic data were analysed using Chromax 2005 application (Pol-Lab, Warsaw).

At the end of the experiment, the rats have been decapitated and their brains taken for histological analysis to confirm that cannulae had been located properly.

*Statistics*

The data are shown as means ±SEM. Data distribution pattern was not normal (unlike Gauss's curve). Statistical analyses for comparing more than two groups nonparametric Kruskal-Wallis's test was used with post-hoc Dunn's test.

RESULTS

*Effect of single administration of nicotine and venlafaxine on the level of neurotransmitters and their metabolites in hippocampus measured in microdialysis in vivo*

*1. Effect of single administration of nicotine and venlafaxine on the level of NA in rats' hippocampus measured in microdialysis in vivo*

Upon single administration of 0.2 mg/kg NIC s.c., a statistically significant increase of NA level was observed in the animals' hippocampus within 20-100 minutes of NIC administration, while within 160-180 minutes a significant decrease of NA level.

Upon single administration of 20 mg/kg VEN i.p., a statistically significant increase of NA level was observed in the animals' hippocampus within 40-180 minutes of administration as compared to the control group and within 40-180 minutes as compared to the NIC group.

Combined administration of NIC and VEN to rats caused statistically significant increase in NA level compared to the control group only in the 20<sup>th</sup> and 80<sup>th</sup> minute of measurement. A statistically significant drop of NA release in rat hippocampus was also observed in comparison to the NIC or the VEN group within 40-120 min., except for the 80<sup>th</sup> and 100<sup>th</sup> minute. Combined administration of NIC and VEN caused statistically significant decrease in NA level compared to the VEN group and increase compared to the NIC group in the 140 - 180<sup>th</sup> minute of measurement (*Fig. 1*).

*2. Effect of single administration of nicotine and venlafaxine on the level of DA in hippocampus measured in microdialysis in vivo*

Upon single administration of 0.2 mg/kg NIC s.c. no statistically significant differences in the DA level were observed.

Upon single administration of 20 mg/kg VEN i.p. to rats, a statistically significant increase of DA level was observed in the animals' hippocampus within 20-100 minutes of administration, with a characteristic peak in the 40<sup>th</sup> minute of measurement, statistically significant as compared to the NIC group.

Combined administration of NIC and VEN caused enhanced release of DA as compared to the control group within 20-160 minutes of measurement and to the NIC group and to the VEN group in the 60-160<sup>th</sup> minutes of measurement (except for the 100<sup>th</sup> minute) (*Fig. 2*).

*3. Effect of single administration of nicotine and venlafaxine on the level of DOPAC in hippocampus measured in microdialysis in vivo*

Upon single administration of NIC, no statistically significant differences in the DOPAC level were observed, except for the 140<sup>th</sup> minute. VEN administration, though, resulted in a progressive, significant decrease of dopamine metabolite - DOPAC - in hippocampus from 80<sup>th</sup> minute on as compared to the control group and between 20-40<sup>th</sup>, 80<sup>th</sup> and 180<sup>th</sup> minute as compared to the NIC group.

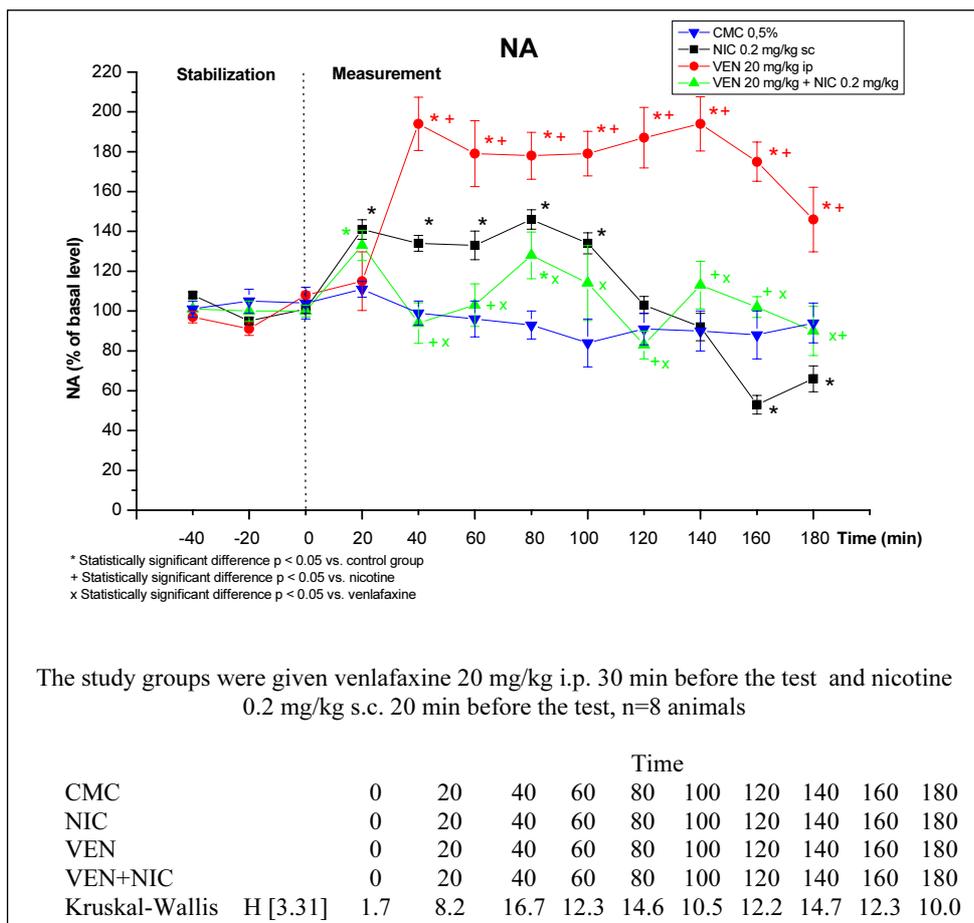


Fig. 1. Effect of single administration of nicotine and venlafaxine on the level of NA in hippocampus measured in microdialysis *in vivo*.

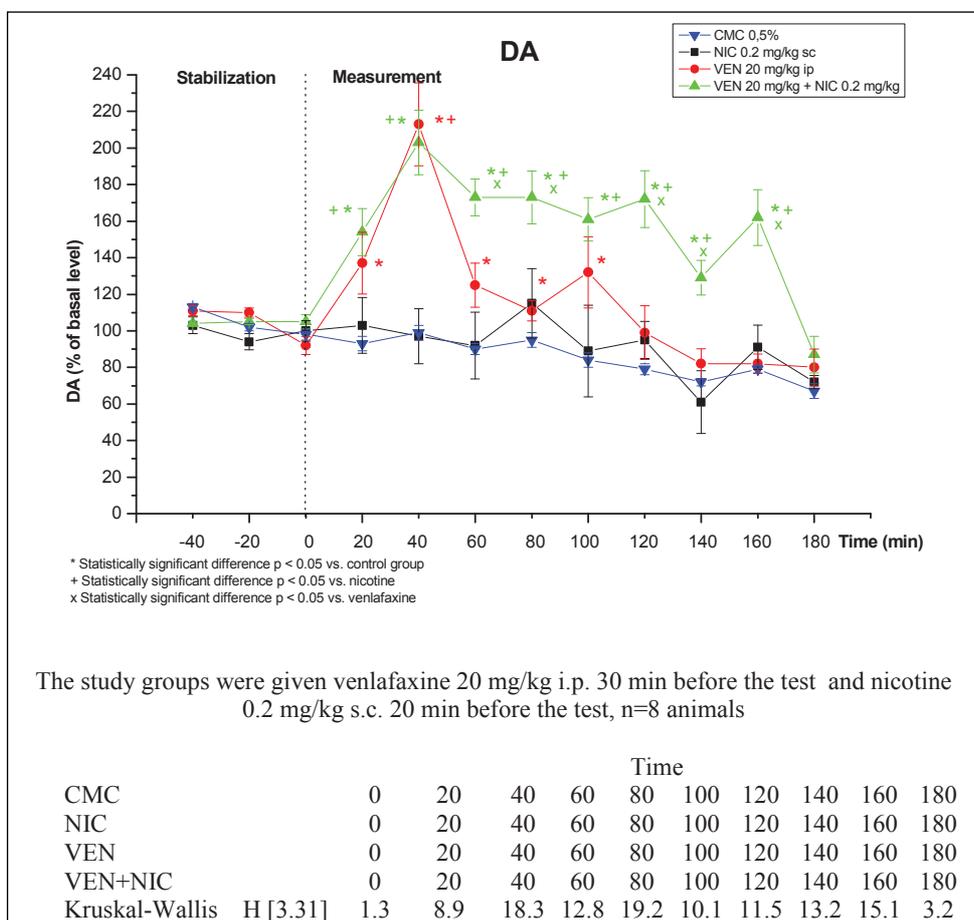


Fig. 2. Effect of single administration of nicotine and venlafaxine on the level of DA in hippocampus measured in microdialysis *in vivo*.

A significant enhancement of DOPAC level decrease was also observed in the VEN+NIC group from the 60<sup>th</sup> minute of measurement on as compared to the control group, and from the 20<sup>th</sup> minute on compared to the NIC group and in the 60<sup>th</sup> and 100-180<sup>th</sup> minute as compared to the VEN group (Fig. 3).

4. Effect of single administration of nicotine and venlafaxine on the level of HVA acid in hippocampus measured in microdialysis in vivo

Upon single administration of NIC, minor variations of HVA acid level were observed as compared to the control group with a statistically significant increase of the metabolite level between 20-40<sup>th</sup> minute and decrease of the metabolite level in the 140<sup>th</sup> and the 180<sup>th</sup> minute of measurement.

Upon VEN administration, HVA acid level decreased compared to the control (between 40-180<sup>th</sup> - except for 60<sup>th</sup> and 80<sup>th</sup> minute of measurement) and the NIC group (between 40-180<sup>th</sup> - except for 60<sup>th</sup>, 80<sup>th</sup> and 140<sup>th</sup> minute of measurement).

Combined administration of NIC and VEN caused enhanced decrease of HVA level in the rats' hippocampus as compared to the control group from the 40<sup>th</sup> minute of measurement and to the NIC group (20-40<sup>th</sup> min and 100-180<sup>th</sup> min) and to the VEN group (60-80<sup>th</sup> min and 120-140<sup>th</sup> min) (Fig. 4).

5. Effect of single administration of nicotine and venlafaxine on the level of 5-HT in hippocampus measured in microdialysis in vivo

Upon single administration of 0.2 mg/kg NIC s.c., an increase of 5-HT level in the hippocampus was observed

between 40-80<sup>th</sup> min of measurement, followed by a decrease of the neurotransmitter level between 140-180<sup>th</sup> minute.

Upon single administration of VEN to rats, increase of 5-HT level was observed in the animals' hippocampus after 40 minutes of administration and for 180 minutes as compared to the control group and NIC group (except for the 80<sup>th</sup> minute), where the highest neurotransmitter level would be observed between 40<sup>th</sup> and 60<sup>th</sup> minute of measurement.

With combined administration of NIC and VEN, an increased release of 5-HT was observed as compared to separate administration of both agents, and this increased level persisted throughout the measurement period, where the highest neurotransmitter level would be observed between 40<sup>th</sup> and 100<sup>th</sup> minute (Fig. 5).

6. Effect of single administration of nicotine and venlafaxine on the level of 5-HIAA acid in hippocampus measured in microdialysis in vivo

Upon single administration of NIC, no statistically significant differences in the 5-HIAA acid level were observed as compared to the control group.

Upon VEN administration, successive decrease in 5-HIAA acid level compared to the control group was observed from the 60<sup>th</sup> min of measurement and compared to the NIC group in the 40<sup>th</sup> min and between 80<sup>th</sup>-180<sup>th</sup> minute of measurement.

Upon combined VEN+NIC administration, a statistically significant decrease of 5-HIAA acid level was observed as compared to the control group (from the 100<sup>th</sup> min of measurement on) and compared to the NIC group (the 40<sup>th</sup> and 100-180<sup>th</sup> min) (Fig. 6).

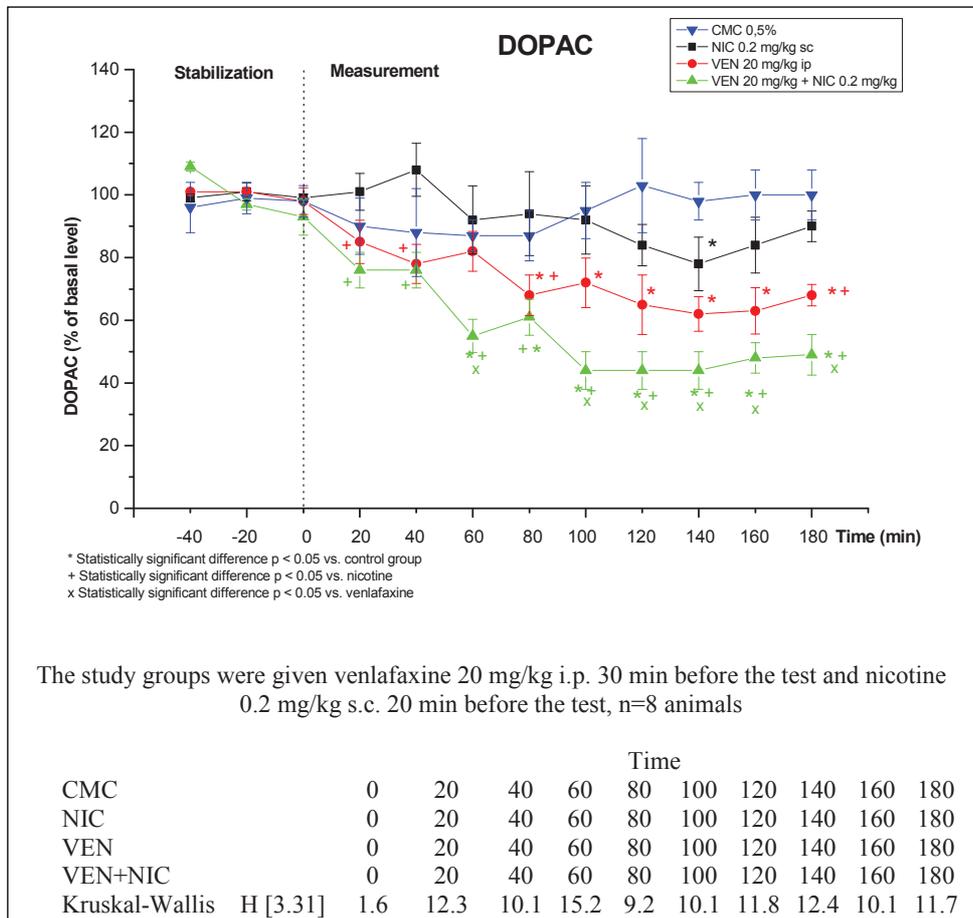


Fig. 3. Effect of single administration of nicotine and venlafaxine on the level of DOPAC in rats' hippocampus measured in microdialysis in vivo.

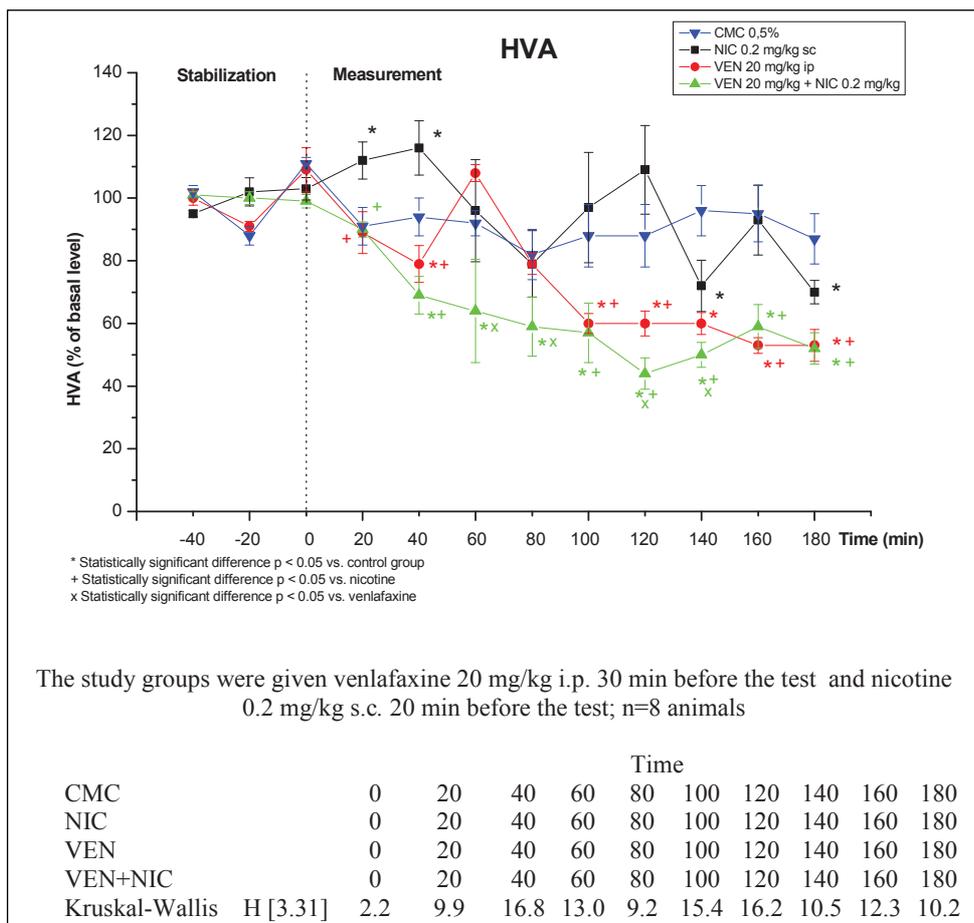


Fig. 4. Effect of single administration of nicotine and venlafaxine on the level of HVA acid in hippocampus measured in microdialysis *in vivo*.

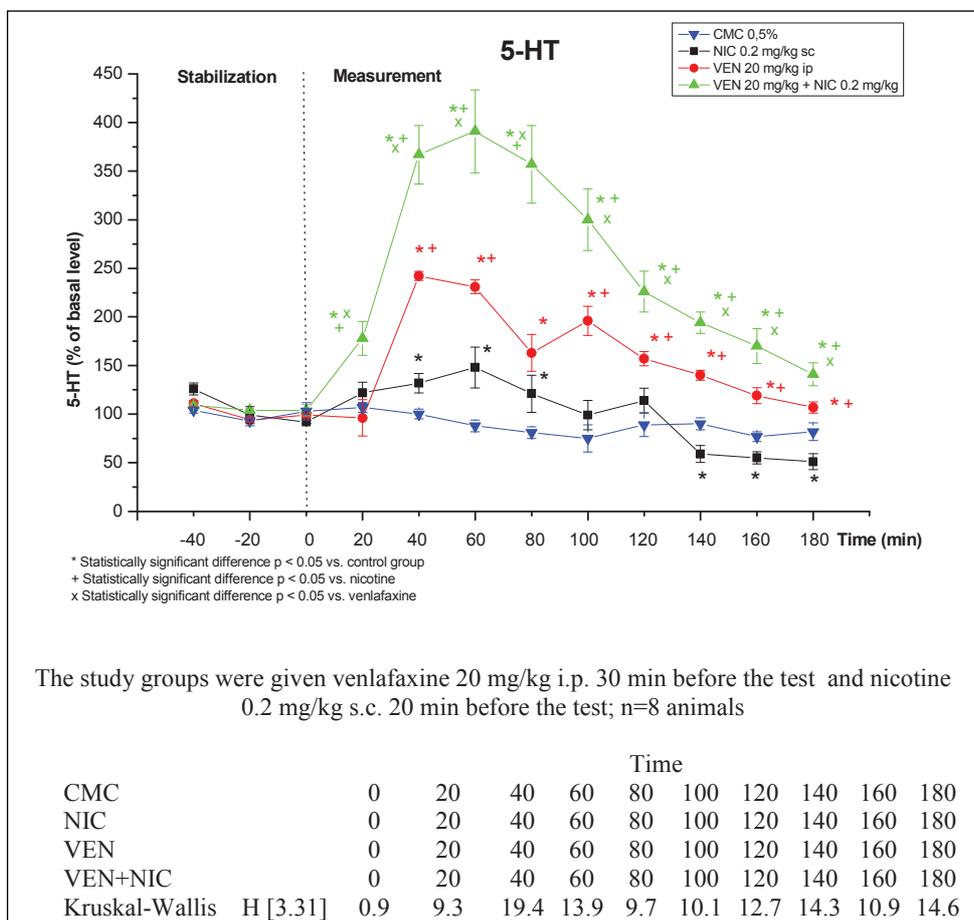


Fig. 5 Effect of single administration of nicotine and venlafaxine on the level of 5-HT in hippocampus measured in microdialysis *in vivo*.

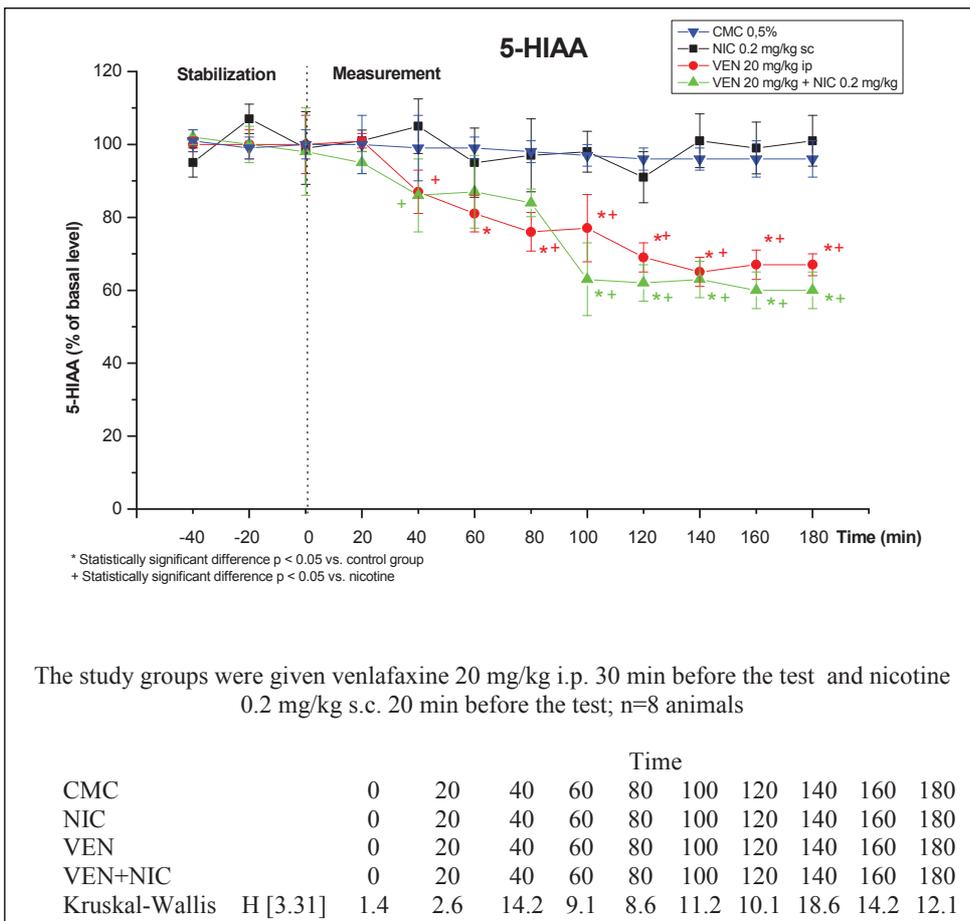


Fig. 6. Effect of single administration of nicotine and venlafaxine on the level of 5-HIAA acid in hippocampus measured in microdialysis *in vivo*.

DISCUSSION

Depression is undoubtedly related to insufficient monoaminergic neurotransmission in the CNS (18, 19), and the use of antidepressants is aimed at restoring the normal neurotransmitter levels (5-HT, NA and DA) in patients with depression.

In our previous studies, VEN administered in the dose of 20 mg/kg has shown antidepressant activity in rats in the Porsolt's test with only single administration, and this effect was maintained with repeated administration (5, 7).

Numerous studies suggest that apart from insufficient monoaminergic transmission, pathogenesis of depression also involves central nicotinic receptors, and NIC has an antidepressant effect through its agonist activity on these receptors (20-23). This thesis may be confirmed by the fact that mecamylamine - an antagonist of nicotinic receptors - neutralises NIC's antidepressant effect (8). It is speculated that apart from the agonist activity on the central nicotinic receptors, NIC's ability to release some neurotransmitters in the CNS (such as NA, 5-HT, DA) believed to be of relevance in generating depression may largely contribute to NIC's antidepressant effect (24). It is also known that beside stimulating receptors NIC also inhibits activity of MAO - an enzyme responsible for serotonin, noradrenaline and dopamine metabolism (23).

As it had been assumed, a significant increase of 5-HT and NA level in the animals' hippocampus was observed following single administration of VEN.

Interaction with the dopaminergic system may be of some importance for the memory improvement observed with VEN administration. It is proved by the increase of the total DA level and decrease in DA metabolites level (DOPAC and HVA) in rats'

hippocampus upon administration of 20 mg/kg VEN i.p. Having in mind that only very large doses of VEN lead to DA reuptake inhibition, it may be assumed that the observed DA level increase is related to another mechanism than this neurotransmitter's reuptake inhibition.

The changes in neurotransmitter levels in the rats' hippocampus upon NIC administration was observed by Rossi *et al.* (25). He reported that NIC administered to rats in the dose of 0.3 mg/kg s.c. induced increase of DA and its metabolites levels in the hippocampus, decrease of 5-HT level and no changes in NA level. The use of antagonists of nicotinic receptors (mecamylamine), muscarinic receptors (atropine), D<sub>1</sub> receptors (SCH23390) and D<sub>2</sub> receptors (eticlopride) neutralises the observed DA increase following NIC administration, while the effect of these agents on 5-HT and NA levels was minor (25). Based on this, the author assumes that the mechanism of DA release by NIC is taking place with the contribution of nicotinic and muscarinic receptors located at DA-ergic endings within the hippocampus, while NIC's effect on NA and 5-HT levels depends on the effect on other receptors in other brain regions other than hippocampus (25).

As opposed to Rossi *et al.* (25), in the presented tests the highest NA level following administration of 0.2 mg/kg NIC was observed within 20-80 minutes of administration. 5-HT level also increased between the 20<sup>th</sup> and 80<sup>th</sup> minute, and decreased after the 140<sup>th</sup> minute as compared to the control group. Sershen *et al.* (26) claims that NA release by NIC occurs *via* nicotinic receptors located on noradrenergic endings.

Kiianamaa *et al.* (27) reported that NIC administered to rats in the dose of 0.25 mg/kg, 0.5 mg/kg and 0.75 mg/kg s.c. induced increase of extracellular DA, DOPAC and HVA levels in the limbic system which is an evidence of dopamine release

stimulation by NIC (27). In other studies, HVA level has shown no significant deviations as compared to the control group, while the level of DOPAC - another dopamine metabolite - decreased (28). The total level of dopamine and serotonin metabolites (DOPAC, HVA, 5-HIAA) in this study were lowered than in the control group.

Surprisingly enough, single administration of 0.2 mg/kg NIC s.c. did not induce major changes in the dopamine level in rats' hippocampus. Maybe the subcutaneous dose of NIC applied was too small compared to the ones used by Kiianamaa *et al.* (27) to result in changes in the dopamine levels in the studied rats. This assumption seems to find confirmation in the paper of Shearman *et al.* (29) where DA level in hippocampus of dialysed rats decreased upon administration of a low dose of NIC (1  $\mu$ M). Note that the NIC dose used by the author was smaller than the one reaching a smoker's brain with one cigarette.

The size of dose applied has a major effect on the obtained results. For instance, NIC administered in the dose of 1 mg/kg increases DOPAC level, which was proved in the experiment conducted by Kubo *et al.* (30) and by applying larger NIC doses (3 and 12 mg/kg) Kiianamaa *et al.* (27) observed a decrease of NA level in frontal cortex but not in other brain regions.

Apart from the size of dose applied, the route of NIC administration is equally important. Mitchell *et al.* (31) found the systemic administration of NIC to increase NA levels while administration directly into the hippocampus had no effect on levels of this neurotransmitter.

In our previous studies (7) we have shown that combined administration of NIC and VEN enhanced the antidepressant effect as compared to separate administration of these substances. Analysis of results of neurotransmitters and their metabolite level measurements upon combined administration of VEN and NIC suggests that the observed potentialisation of VEN's procognitive effect may be tried to be explained by the increase in 5-HT and DA levels and decrease of their metabolite levels (5-HIAA, HVA and DOPAC) in hippocampus. Interestingly, it turns out that the NIC dose of 0.2 mg/kg (too small to observe DA level increase in rat's hippocampus upon single administration) is sufficient to cause major changes in dopaminergic activity in this brain region upon combined administration with VEN. DA level determined in the 40<sup>th</sup> minute of measurement (it was then that the highest DA levels were reported for both VEN and VEN+NIC groups) was slightly higher in rats receiving VEN. The long-lasting high DA level in hippocampus of dialysed rats suggests the possibility of potentialisation of VEN's behavioural effects by NIC. The total NA level in this experiment was lower in the combined group (VEN+NIC) than in the VEN-only group. This is probably an evidence of lower importance of NIC's interaction with the noradrenergic system for VEN's antidepressant effect. Moreover, both drugs have similar indirect mechanisms of action (they both increase NA and 5-HT in the synaptic cleft) and this may be responsible for observed in our experiments lack of synergy, since synergy more likely may be expressed after co-administration of two drugs with diverse mechanism of action (32). Results presented herein also indicate a major increase in 5-HT level and decrease in its metabolite level (5-HIAA) upon combined administration of VEN and NIC, which explains the enhanced procognitive effect of VEN observed by us in the Morris test.

## CONCLUSION

It may be stated that both drugs release 5-HT and NA, but VEN to a greater degree. DA level was affected only by VEN, however NIC extended the DA system's response to VEN's effect.

Combined administration of drugs resulted in the greatest changes in the 5-HT system. Both drugs contributed to reduction in the neurotransmitter biotransformation.

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