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Antidepressant and anxiolytic efficacy of single, chronic and concomitant use of vortioxetine, dapoxetine and fluoxetine in prenatally stressed rats

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Depression is a highly prevalent social disease. Despite significant medical progress, therapeutic solutions for optimising treatment of this disease are still being sought. The aim of this study was to assess, using the forced swimming test, locomotor activity test and two compartment exploratory test, for a reduction in immobility time (a measure of anti-depressant efficacy), locomotor activity and anxiolytic effectiveness after single, repeated, and combined administration of vortioxetine (2.5 mg/kg – a multimodal SMS), dapoxetine (3.0 mg/kg – an SSRI used in premature ejaculation disorders) and fluoxetine (5.0 mg/kg – an SSRI) in non-stressed and prenatally stressed rats. It was found that vortioxetine, fluoxetine and dapoxetine reduced immobility time and rat locomotor activity which suggests anti-depressant efficacy of these drugs both in monotherapy and in combined administration. The results also confirmed an anxiolytic effect of the study drugs in mono and combined therapy. Analysis of the pathomechanism of depression and the mechanisms of action of the individual drugs tested resulted in a prediction that combined administration of these drugs may be effective in the treatment of depressive disorders, although possible interactions between the drugs used must be assessed for. Considering the fact that dapoxetine is not currently used in depression treatment and vortioxetine is a relatively new drug, further research in this direction is vital, including within animal models.

Key words: vortioxetine, dapoxetine, fluoxetine, SSRI, forced swimming test, anxiety

INTRODUCTION

Depression is one of the most common and most disabling mental disorders characterised by a major burden on both the patients and society as a whole. The most common symptoms of depression include low mood, low self-esteem, sleeping disorders and anxieties, which together frequently lead to suicides. It should be noted that the incidence of both (depression and anxiety disorders) have been on the rise, which may be related to longer lifespans, unemployment, migrations, sociopolitical relationships, and consumption of various chemical compounds (Sidorchuk et al., 2017). Mechanisms responsible for these disorders primarily concern the modulation of monoamines level (serotonin, dopamine, noradrenaline) in CNS structures (Russo and Nestler, 2012), i.e., prefrontal and temporal cortex, limbic structures (hippocampus and amygdaloid body), and basal nuclei (globus pallidus, subthalamic nucleus, black matter, and striatum with caudate nucleus), which are responsible for formation of frontal-subcortical connections ensuring control of functions such as movement, motivation and reward mechanisms (Pandya et al., 2012). Moreover, neurobiological tests have found that hypothalamus-pituitary-adrenal

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axis regulation disorders can significantly contribute to the development of depression (Maletic et al., 2007). Exposure to stress causes the hypothalamus to release corticotropin releasing hormone (CRH), which stimulates the pituitary to synthesize and release adrenocorticotropic hormone (ACTH). ACTH, in turn, is responsible for stimulation of adrenal cortex cells and release of the stress hormone cortisol (corticosterone in animals). This release can cause a number of adverse effects, including damage to neurons in the hippocampus and prefrontal cortex (Lucassen et al., 2014), a reduction in the level of brain-derived neurotrophic factor (BDNF), and restricted neurogenesis (Kinnunen et al., 2003). The prenatal stress model used in this study, based on an induced increase in corticosterone levels (Van den Hove et al., 2005), is an effective method for achieving an animal model of depression.

Polypharmacotherapy, i.e., the combined use of drugs, is frequently utilized in the treatment of depression. Usually, the effects of an anti-depressant agent are discernable after 2–4 weeks of pharmacotherapy (Machado-Vieira et al., 2010), which is why they are increasingly used in combination therapy in an attempt to achieve the effect earlier. Apart from offering undoubted advantages, polypharmacotherapy also entails the risk of a number of adverse effects; thus, special care should be exercised, particularly when combining drugs of the same receptor class.

Vortioxetine (VOR) is a newer anti-depressant that is used for severe forms of depression and entered the market in 2013. Its mechanism of action is multifunctional depending on the location of action, as it stimulates serotonin receptors directly or inhibits serotonin reuptake (Pae et al., 2015). Fluoxetine (FLU), on the other hand, is a 2nd generation antidepressant, selectively inhibiting serotonin reuptake by blocking serotonin transporter (SERT) inhibitor. FLU is used mainly in mild and moderately advanced forms of depression (Guest et al., 2004). Dapoxetine (DAP), although chemically a selective serotonin reuptake inhibitor (SSRI), is not used to treat depression but has been successfully used in pharmacotherapy for premature ejaculation in men. DAP is the first and only drug approved for treatment of premature ejaculation in males aged 18–64 years (McCarty and Dinsmore, 2012).

This study's objective was to investigate for a reduction in immobility time (measure of anti-depressant efficacy), anxiolytic effectiveness and locomotor activity patterns after single, chronic and combined administration of VOR, DAP, or FLU to non-stressed and prenatally stressed rats. In accordance with a generally adopted methodology (Porsolt et al., 1978) and the experience of the investigators (Nowakowska et al., 2014; Ratajczak et al., 2013; 2017), the anti-depressant properties of the study substances were analyzed using the results of the forced swimming test. Nevertheless, we would like to point out that an increasing number of scientific reports (de Kloet and Molendijk, 2016; Molendijk and de Kloet, 2015; Stepanichev et al., 2016) suggest that the forced swimming test is a study tool designed solely for analysis of animal activity changes (active to passive behavior) when faced with a stress factor which, in this case, is a water-filled cylinder and staying in a non-standard environment during the test. Considering the large number of published research papers both confirming and denying the possible application of the forced swimming test to study anti-depressant properties of drugs, we leave this issue open.

METHODS

Animals

Pregnant animals (76 female Wistar rats) were housed individually in cages (42 x 26 cm) in a light- (lights on 07.00–19.00 h), temperature- and humidity-controlled animal facility. Offspring male rats (216 Wistar rats) were housed in similar conditions. The dams had free access to rat chow (Labofeed B) and water.

For behavioral tests, adult male rats born to dams exposed to prenatal stress and male rats born to non-stressed dams (control group) were used. The pregnant females were stressed outside of the experimental box.

The total number of study animals was 292 (76 females, 216 males). The male rats were the offspring of either 38 prenatally stressed females (that delivered 108 prenatally-stressed (PS) group rats) or 38 non-stressed females (that delivered 108 non-stressed control (NSC) group rats). To prevent an effect of litter, only one pup from each litter was used.

Study groups: forced swimming test – 36 NSC group, 36 PS group; locomotor activity test – 36 NSC group, 36 PS group; two compartment exploratory test – 36 NSC group,36 PS group.

The NSC and PS animals were further divided due to the substance being received: vehicle – NSC (n=6), PS (n=6); vortioxetine – NSC (n=6), PS (n=6); dapoxetine – NSC (n=6), PS (n=6); fluoxetine – NSC (n=6), PS (n=6); vortioxetine + fluoxetine – NSC (n=6), PS (n=6); vortioxetine + dapoxetine – NSC (n=6), PS (n=6).

All procedures related to the use of rats in these experiments were conducted according to the ethical principles regarding experiments on animals (Directive 2010/63/EU), especially in the case of sample size (n=6) which is consistent with the 3R principle (replacement, reduction, refinement).

Drugs

Drugs used in the experiments:
- Vortioxetine VOR (2.5 mg/kg i.p.) – Lundbeck, Poland
- Dapoxetine DAP (3 mg/kg i.p.) – Berlin-Chemie/ Menarini, Poland
- Fluoxetine FLU (5 mg/kg i.p.) – Orion Pharma, Poland
- Vehicle – saline group (0.9% Sodium Chloride)

On each experimental day (forced swimming test/ two compartment exploratory test/locomotor activity test) all substances were injected 30 min before the test with either drug or vehicle (i.e., on drug administration days 1, 7, 14 and 21).

Animal model of depression

Pregnancy was determined by observation of vaginal plugs (embryonic day 0–E0). Restraint stress was applied daily during the last week of pregnancy, i.e., the third trimester (E14–E21). Pregnant female rats were individually restrained three times a day (at approximately 9:00 a.m., 1:00 p.m. and 5:00 p.m.) for 45 min in metal tubes (30 cm × 10 cm × 8 cm), while at the same time being exposed to bright light (Van den Hove et al., 2005). Control (NSC) pregnant females were left undisturbed in their home cages.

Forced swimming test

Thirty minutes after drug administration, the rats were placed in cylinders (50 cm height, 19 cm diameter containing water (25°C) and immobility was measured for 5 min (after the pretest - 24 h prior to the experiments, the rats were individually placed in plexiglass cylinders containing water. Fifteen minutes later, the rats were removed to a 30°C drying room for 30 min). A rat was judged to be immobile when it remained floating in the water in an upright position and only made very small movements necessary to keep its head above water. The total duration of immobility over the 5 min period was recorded by an observer unaware of the treatment group of the rat (Porsolt et al., 1978).

Locomotor activity

Locomotor activity was measured in an IR Motor Activity Monitor (Model LE8825, Panlab Harvard Apparatus). On the day of the experiments, the animals were placed individually into the experimental cage (45 cm × 45 cm). During a 5 min observation the following parameters were recorded by photocells and the ActiTrack program (Panlab Harvard Apparatus): locomotion, global activity, stereotypies, maximum speed, distance traveled, resting time and movement speed. The test provided an index of basal locomotor activity for the animals in a test field.

Two compartment exploratory test

Anxiolytic-like effects (anxiety-related behavior) were assessed for in a two-compartment exploratory test. The apparatus employed to test "approach-avoidance behavior" was a conventional open field (100 cm × 100 cm) with a white floor divided into 25 (5 cm × 5 cm) equal squares by a black grid. This surface was divided into two different compartments. One compartment consisted of a squared area (40 cm × 40 cm) in one corner of the open field, with all the surfaces blackened and a roof fitted 35 cm from the floor to prevent light (100 lux lamp) from entering from above, and the second compartment consisted of the remaining white part of the open field, which was uniformly lit by a fluorescent lamp. At the beginning of the test, the rat was placed in the white area in the corner of the compartment. The number of transitions between the two-compartments (BWT), square entries in the black compartment (BSE) and square entries in the white compartment (WSE) were recorded for a 5-min period (Crawley and Goodwin, 1980). Only entries in the white compartment where shown in the results. An event was recorded whenever the rat crossed a line on the grid or the compartment border with all four legs.

Statistical analysis

The data are shown as the mean values ± SEM. The data distribution pattern was not normal (unlike a Gaussian function). Statistical analyses for the forced swimming test and locomotor activity test were carried out using the nonparametric Kruskal-Wallis H test for unpaired data and ANOVA Friedman two-way analysis of variance test for paired data. Statistical significance was tested using Dunn's *post-hoc* test.

RESULTS

The effects of single and chronic treatments with vortioxetine, dapoxetine and fluoxetine and the effects of combined administration on immobility time (IT) as analysed in the Porsolt test on non-stressed and prenatally stressed rat

Comparison of NSC and PS vehicle groups showed that PS animals on day 7 and 14 of the test exhibited

a statistically significant higher immobility time in the Porsolt test, confirming a depression-inducing effect of prenatal stress and validating the animal model of depression used in this study (Table I).

Single administrations of VOR (2.5 mg/kg i.p.), DAP (3 mg/kg i.p.), or FLU (5 mg/kg i.p.) failed to cause a statistically significant difference between immobility time in the NSC and vehicle group. A statistically significant effect (reduced immobility time) was observed upon chronic treatment (14 and 21 days) of VOR (2.5 mg/kg i.p.) (p<0.05 *vs.* NSC vehicle group), chronic treatment (21 days) of DAP (3 mg/kg i.p.) (p<0.05 *vs.* NSC vehicle group), and chronic treatment (14 and 21 days) of FLU (5 mg/kg i.p.) compared to the vehicle group (p<0.05 *vs.* NSC vehicle group) (Table I).

Combined VOR+FLU and DAP+FLU administration in the NSC group resulted in a decrease in immobility time as early as the first dose in comparison to the vehicle group (p<0.05 *vs.* NSC vehicle group) and compared to the administration of VOR (p<0.05, VOR+FLU *vs.* NSC VOR) or FLU (p<0.05, VOR+FLU *vs.* NSC FLU) alone and DAP (p<0.05, DAP+FLU *vs.* NSC DAP) or FLU (p<0.05, DAP+FLU *vs.* NSC FLU) alone in the NSC groups. This effect, however, failed to hold upon chronic treatment (Table I).

In the PS group, chronic treatment (7, 14, and 21 days) of VOR at a dose of 2.5 mg/kg i.p. 30 minutes prior to the test resulted in a statistically significant reduction in immobility time compared to the vehicle group (p<0.05 *vs.* PS vehicle group). Similar results were obtained upon chronic treatment (14 and 21 days) of

Table I. Effect of single and chronic treatment of VOR, DAP, and FLU and combined administration on immobility time (IT) analysed in the Porsolt test on non-stressed and prenatally stressed rats.

Group	Immobility Time (IT) [s]				Friedman H [3.23]
	Single treatment	Chronic treatment			
		7 days	14 days	21 days	
Non-stressed control (NSC)					
Vehicle	193.20 ± 14.15	253.00 ± 10.96	265.60 ± 10.40	283.16 ± 7.11	8.5
VOR 2.5 mg/kg *ip* 30 min before the test	190.40 ± 10.07	260.33 ± 6.78	236.00 ± 6.28*	257.00 ± 7.50*	8.9
DAP 3 mg/kg *ip* 30 min before the test	195.20 ± 14.82	245.40 ± 11.21	251.60 ± 9.34	261.40 ± 8.58*	5.8
FLU 5 mg/kg *ip* 30 min before the test	220.20 ± 13.01	260.80 ± 7.97	241.80 ± 7.99*	269.40 ± 5.68*	5.3
VOR 2.5 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	140.50 ± 12.90*[ac]	261.80 ± 13.20	258.16 ± 11.39	264.40 ± 7.95	10.7
DAP 3 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	144.50 ± 9.33*[bc]	267.20 ± 11.72	261.60 ± 8.16	267.00 ± 7.24	13.2
Prenatally-stressed (PS)					
Vehicle	172.80 ± 7.52	273.00 ± 7.37*	286.20 ± 1.62*	282.66 ± 2.66	18.2
VOR 2.5 mg/kg *ip* 30 min before the test	168.00 ± 9.08	241.66 ± 9.54[X]	250.33 ± 8.75[X]	272.83 ± 3.79[X]	12.8
DAP 3 mg/kg *ip* 30 min before the test	138.70 ± 13.70[X]	249.80 ± 8.80[X]	248.40 ± 11.09[X]	275.26 ± 8.80	12.6
FLU 5 mg/kg *ip* 30 min before the test	179.40 ± 7.35	265.16 ± 10.57	250.00 ± 14.34[X]	271.00 ± 3.64[X]	9.3
VOR 2.5 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	106.00 ± 11.52[Xac]	249.33 ± 8.22 [Xc]	261.66 ± 6.15[X]	258.00 ± 8.69[Xa]	17.5
DAP 3 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	147.40 ± 14.29	234.33 ± 7.86[X]	246.60 ± 11.39[X]	264.66 ± 7.64 [X]	11.0
Kruskal Wallis H [11.71]	9.7	4.0	5.3	3.8	

n=6; * Statistically significant difference p<0.05 *vs.* NSC vehicle; [X] Statistically significant difference p<0.05 *vs.* PS vehicle; [a] Statistically significant difference p<0.05 *vs.* VOR; [b] Statistically significant difference p<0.05 *vs.* DAP; [c] Statistically significant difference p<0.05 *vs.* FLU.

FLU at a dose of 5 mg/kg compared to the vehicle group (p<0.05 *vs.* PS vehicle group). For DAP (3 mg/kg i.p.), reduced immobility time was observed both upon single and chronic treatment (7 and 14 days) (p<0.05 *vs.* PS vehicle group) (Table I).

In the case of combined VOR+FLU administration, an anti-depressant effect was observed after the first dose and upon chronic treatment (7, 14, and 21 days) as compared to the vehicle group (p<0.05 *vs.* PS vehicle group) and single administration of VOR (p<0.05 *vs.* PS VOR) or FLU (1 and 7 days, p<0.05 *vs.* PSG FLU), while the combined administration of DAP+FLU only resulted in an anti-depressant effect upon chronic treatment (7, 14, and 21 days) compared to the vehicle group (p<0.05 *vs.* PS vehicle group (Table I).

The effects of single and chronic treatment of vortioxetine, dapoxetine, and fluoxetine and the effects of combined administration on locomotor activity of non-stressed and prenatally stressed rat

A statistically significant effect (reduced locomotor activity, LA) was observed upon chronic treatment (21 days) with VOR (2.5 mg/kg i.p.) (p<0.05 *vs.* NSC vehicle group), chronic treatment (14 and 21 days) with DAP (3 mg/kg i.p.) (p<0.05 *vs.* NSC vehicle group), and chronic treatment (14 and 21 days) with FLU (5 mg/kg i.p.) compared to the vehicle group (p<0.05 *vs.* NSC vehicle group) demonstrated a sedative effect of the drugs upon chronic treatment (Table II).

Table II. Effect of single and chronic treatment of VOR, DAP, and FLU and combined administration on locomotor activity of non-stressed and prenatally stressed animals.

Group	Activity counts				Friedman H[3.23]
	Single treatment	Chronic treatment			
		7 days	14 days	21 days	
Non-stressed control (NSC)					
Vehicle	731.50 ± 48.70	672.66 ± 57.48	593.83 ± 60.90	632.00 ± 54.40	2.4
VOR 2.5 mg/kg *ip* 30 min before the test	769.16 ± 59.66	594.5 ± 62.06	468.83 ± 50.68	397.50 ± 50.11*	6.6
DAP 3 mg/kg *ip* 30 min before the test	815.33 ± 77.51	555.00 ± 58.83	254.00 ± 22.88*	316.50 ± 54.75*	9.8
FLU 5 mg/kg *ip* 30 min before the test	747.16 ± 53.71	569.00 ± 79.78	334.50 ± 54.67*	357.16 ± 46.86*	6.8
VOR 5 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	613.66 ± 40.31[a]	644.83 ± 48.46	301.83 ± 54.58*[a]	322.16 ± 41.44*	7.4
DAP 3 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	477.66 ± 54.31*[bc]	468.5 ± 63.71*	202.33 ± 28.75*	196.00 ± 34.59*[c]	5.9
Prenatally-stressed (PS)					
Vehicle	788.66 ± 21.92	531.66 ± 57.73	655.00 ± 44.78	594.16 ± 54.60	5.4
VOR 2.5 mg/kg *ip* 30 min before the test	898.00 ± 64.32	425.83 ± 37.99	581.50 ± 28.70	280.16 ± 50.97[X]	13.0
DAP 3 mg/kg *ip* 30 min before the test	934.16 ± 39.76[X]	558.16 ± 33.91	440.33 ± 58.87[X]	330.00 ± 48.09[X]	10.1
FLU 5 mg/kg *ip* 30 min before the test	841.83 ± 69.40	456.00 ± 53.82	599.50 ± 69.20	375.00 ± 59.36[X]	7.3
VOR 5 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	338.33 ± 53.27[Xac]	230.16 ± 33.35[Xac]	207.50 ± 40.08[Xac]	269.83 ± 59.80[X]	2.7
DAP 3 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	564.83 ± 47.37[Xbc]	234.83 ± 42.15[Xbc]	196.50 ± 41.28[Xbc]	388.83 ± 62.40[X]	7.5
Kruskal-Wallis H [11.71]	10.9	8.1	9.5	8.4	

n=6; * Statistically significant difference p<0.05 *vs.* NSC vehicle; [X] Statistically significant difference p<0.05 *vs.* PS vehicle; [a] Statistically significant difference p<0.05 *vs.* VOR; [b] Statistically significant difference p<0.05 *vs.* DAP; [c] Statistically significant difference p<0.05 *vs.* FLU.

In the case of NSC rats, combined administration, a significant effect (reduced LA) was observed for chronic treatment (14 and 21 days) with VOR+FLU (p<0.05 *vs.* NSC vehicle group) and for single and repeated (7, 14, and 21 days) administration of DAP+FLU (p<0.05 *vs.* NSC vehicle group). These results show that a sedative effect occurred with the combined administration of drugs relative to the NSC group (Table II).

Moreover, in the NSC group, a statistically significant LA reduction was shown for both single and chronic treatment (14 days) with VOR+FLU compared to the administration of VOR alone (p<0.05 *vs.* NSC VOR). A statistically significant LA reduction was also observed upon single administration of DAP+FLU compared to a single administration of either DAP (p<0.05 *vs.* NSC VOR) or FLU (p<0.05 *vs.* NSC FLU) alone. A similar effect for the combined administration of DAP+FLU was seen in the case of chronic treatment – a LA reduction was observed upon chronic treatment with these drugs (21 days) compared to the FLU administration (p<0.05 *vs.* NSC FLU) (Table II).

In the PS group, a statistically significant reduction in LA in animals (sedative effect) was observed upon chronic treatment with VOR at a dose of 2.5 mg/kg (21 days), DAP at a dose of 3 mg/kg (14 and 21 days), and FLU at a dose of 5 mg/kg (21 days) compared to the vehicle group (p<0.05 *vs.* PS vehicle group). A single administration of DAP, on the other hand, had an opposite effect, i.e., it increased LA (stimulant effect) compared to the vehicle group (p<0.05 *vs.* PS vehicle group) (Table II).

Table III. Effects of single and chronic administration of VOR, DAP, and FLU on reducing the anxiety like behavior in the non-stressed and prenatally stressed rats.

Group	Entries in the white compartment				Friedman H [3.23]
	Single treatment	Chronic treatment			
		7 days	14 days	21 days	
Non-stressed control (NSC)					
Vehicle	7.00 ± 1.15	9.50 ± 1.45	7.83 ± 0.79	7.83 ± 1.24	2.4
VOR 2.5 mg/kg *ip* 30 min before the test	19.83 ± 4.46*	24.00 ± 2.03*	20.17 ± 2.72*	14.83 ± 1.37*	5.1
DAP 3 mg/kg *ip* 30 min before the test	12.16 ± 1.30	11.50 ± 1.61	9.33 ± 0.88	8.50 ± 1.33	2.8
FLU 5 mg/kg *ip* 30 min before the test	14.17 ± 1.53*	12.83 ± 1.07*	16.00 ± 1.75*	14.17 ± 1.44*	2.3
VOR 2.5 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	8.66 ± 1.54^{ac}	12.33 ± 1.90^{a}	6.33 ± 1.02^{ac}	5.83 ± 1.35^{ac}	6.7
DAP 3 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	5.33 ± 1.33^{bc}	8.16 ± 1.02	12.83 ± 2.12*	12.33 ± 1.28*	9.3
Prenatally-stressed (PS)					
Vehicle	3.50 ± 0.56*	4.83 ± 0.79*	4.83 ± 0.74*	3.83 ± 0.71*	2.1
VOR 2.5 mg/kg *ip* 30 min before the test	18.50 ± 4.13^{X}	15.33 ± 1.45^{X}	11.83 ± 1.62^{X}	13.00 ± 1.36^{X}	3.8
DAP 3 mg/kg *ip* 30 min before the test	8.03 ± 3.01^{X}	7.16 ± 0.79^{X}	7.16 ± 0.90^{X}	6.33 ± 0.84^{X}	1.8
FLU 5 mg/kg *ip* 30 min before the test	17.33 ± 1.89^{X}	20.83 ± 3.75^{X}	14.83 ± 1.74^{X}	14.50 ± 1.23^{X}	4.9
VOR 2.5 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	5.50 ± 1.26^{Xac}	7.66 ± 0.95^{Xac}	9.16 ± 1.37^{Xc}	6.66 ± 1.02^{Xac}	2.0
DAP 3 mg/kg *ip* FLU 5 mg/kg *ip* 30 min before the test	3.00 ± 0.26^{bc}	6.16 ± 1.47^{c}	8.83 ± 1.24^{Xc}	8.83 ± 1.97^{Xc}	7.1
Kruskal Wallis H TOTAL [11.71]	10.8	9.9	10.0	8.9	

n=6; * Statistically significant difference p<0.05 *vs.* NSC vehicle; ^{X} Statistically significant difference p<0.05 *vs.* PS vehicle; ^{a} Statistically significant difference p<0.05 *vs.* VOR; ^{b} Statistically significant difference p<0.05 *vs.* DAP; ^{c} Statistically significant difference p<0.05 *vs.* FLU.

In the case of combined administration of the drugs in PS rats, a statistically significant reduction in LA in animals (sedative effect) was observed upon both single and repeated (7, 14, and 21 days) administration of combined VOR+FLU and DAP+FLU compared to the vehicle group ($p<0.05$ *vs.* PS vehciel group) (Table II).

Moreover, in the PS group, a statistically significant reduction in LA (sedative effect) was observed for combined administration of VOR+FLU (1, 7, and 14 days) compared to administration of VOR ($p<0.05$ *vs.* PS VOR) or FLU ($p<0.05$ *vs.* PS FLU) alone. A reduction in LA was also observed in the group receiving combined administration of DAP+FLU (1, 7, and 14 days) compared to administration of DAP ($p<0.05$ *vs.* PS DAP) or FLU ($p<0.05$ *vs.* PS FLU) alone (Table II).

The effects of single and chronic administration of vortioxetine, dapoxetine and fluoxetine on reducing the anxiety-like behavior in the non-stressed and prenatally stressed rat

A comparison between the NSC and PS vehicle-injected groups revealed a statistically significant decrease in number of entries into the white compartment of the two-compartment exploratory test (1, 7, 14 and 21 days of observation) in PS rats compared to NSC rats ($p<0.05$ *vs.* NSC vehicle group) demonstrating an anxiogenic effect of prenatal stress and validating this animal of depression (Table III).

Single and chronic treatment (1, 7, 14, 21 days) of VOR (2.5 mg/kg i.p.) and FLU (5 mg/kg i.p.) administered to NSC rats produced a statistically significant increase in the number of entries into the white compartment, which is evidence of an anxiolytic effect ($p<0.05$ *vs.* NSC vehicle group) (Table III). The statistically significant increase in the number of entries into the white compartment (anxiolytic effect) was also observed after multiple (14, 21 days) administrations of combined therapy with DAP (3 mg/kg i.p.) + FLU (5 mg/kg i.p.) ($p<0.05$ *vs.* NSC vehicle group) (Table III).

Moreover, NSC rats showed a statistically significant decrease in the number of entries into the white compartment after combined therapy with VOR (2.5 mg/kg i.p.) + FLU (5 mg/kg i.p.) and VOR (2.5 mg/kg i.p.) + DAP (3 mg/kg i.p.) compared to monotherapy with VOR (2.5 mg/kg i.p.) (1–21 days of treatment) ($p<0.05$ *vs.* NSC VOR) (Table III). A statistically significant decrease in the number of entries into the white compartment was also observed after combined therapy with VOR (2.5 mg/kg i.p.) + FLU (5 mg/kg i.p.) (1, 14 and 21 days) and after a single administration of combined therapy with DAP (3 mg/kg i.p.) + FLU (5 mg/kg i.p.) compared to monotherapy with FLU (5 mg/kg i.p.) ($p<0.05$ *vs.* NSC FLU) (Table III). Similar results were obtained after the combined administration of both VOR (2.5 mg/kg i.p.) + DAP (3 mg/kg i.p.) (1 and 14 days) and DAP (3 mg/kg i.p.) + FLU (5 mg/kg) compared to monotherapy with DAP (3 mg/kg i.p.) ($p<0.05$ *vs.* NSC DAP) (Table III). The results showed a better anxiolytic profile for drugs such as vortioxetine and fluoxetine when administered individually (not in combined therapy) in the non-stressed animals.

In the PS group, single and chronic (7, 14, 21 days) treatment with VOR (2.5 mg/kg i.p.), DAP (3 mg/kg i.p.) and FLU (5 mg/kg i.p.) produced a statistically significant increase in the number of entries into the white compartment compared to the vehicle group ($p<0.05$ *vs.* PS vehicle group), which provides evidence that anxiolytic effects were induced by the drugs administered to the prenatally stressed animals (Table III). The increase in the number of entries into the white compartment of the two-compartment exploratory test was also observed after the combined administration of VOR (2.5 mg/kg i.p.) + FLU (5 mg/kg i.p.) (1–21 days) and after multiple administrations of VOR (2.5 mg/kg i.p.) + DAP (3 mg/kg i.p.) (7–21 days) and DAP (2.5 mg/kg i.p.) + FLU (5 mg/kg i.p.) (14–21 days) compared to the vehicle group ($p<0.05$ *vs.* PS vehicle group) (Table III).

Moreover, the results showed a decrease in the number of entries into the white compartment after combined therapy of VOR (2.5 mg/kg i.p.) + FLU (5 mg/kg i.p.) (1, 7 and 21 days) and VOR (2.5 mg/kg i.p.) + DAP (3 mg/kg i.p.) (1 and 14 days) compared to monotherapy with VOR (2.5 mg/kg i.p.) ($p<0.05$ *vs.* PS VOR) (Table III). Similar results were obtained after combined treatment with VOR (2.5 mg/kg i.p.) + FLU (5 mg/kg i.p.) (1–21 days) and DAP (3 mg/kg i.p.) + FLU (5 mg/kg i.p.) (1–21 days) compared to monotherapy with FLU (5 mg/kg i.p.) ($p<0.05$ *vs.* PS FLU) (Table III). Lastly, combined therapy with DAP (3 mg/kg i.p.) + FLU (5 mg/kg i.p.) also resulted in a decrease in the number of entries into the white compartment compared to monotherapy with DAP (3 mg/kg i.p.) ($p<0.05$ *vs.* PS DAP) (Table III). As with the NSC rats, the results demonstrated a better anxiolytic profile for drugs when they were administered individually (not in combined therapy) in the prenatally stressed rats.

DISCUSSION

VOR is, on one hand, an agonist of 5-HT_{1A} and 5-HT_{1B} receptors and, on the other, an antagonist of 5-HT_{1D}, 5-HT_3, and 5-HT_7 serotonin receptors, as well as a SERT inhibitor (D'Agostino et al., 2015). Then FLU, as a typical SSRI, blocks 5-HT_{2A}, 5-HT_{2C} receptors while showing an affinity for SERT (Guest et al., 2004). There are studies confirming FLU's affinity to 5-HT_{1A} receptors (Sub-

hash et al., 2000). DAP, however, shows no affinity for receptors although the agent is a strong SERT inhibitor similarly to VOR and FLU (Artigas 2013). An obvious question arises about potential anti-depressant and anxiolytic mechanisms and the involvement of specific receptor groups in these processes. Artigas (2013), in his general reference paper on serotonin receptors involved in anti-depressant effects, attributes the greatest importance to 5-HT_{1A} receptors while still emphasizing the significance of 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_4, and SERT receptors. He also draws attention to 5-HT_6 and 5-HT_7 receptors, whose role in anti-depressant effects are not entirely understood currently, but previous results indicate that blocking these receptors may potentiate the effect of anti-depressants (Mullins et al., 1999). Anti-depressant and anxiolytic effects may, thus, be achieved through blocking 5-HT_{1A} autoreceptors (none of the study drugs show such effects) or stimulation of 5-HT_{1A} heteroreceptors (VOR and FLU have such an effect) (Subhash et al., 2000; Santana et al., 2004), activation of 5-HT_{1B} receptors (VOR) (Tatarczyńska et al., 2004), inhibition of 5-HT_{2A} and 5-HT_{2C} receptors (FLU) (Santana et al., 2004, Millan, 2005), stimulation of 5-HT_4 receptor (none of the study drugs shows such effects) (Warner-Schmidt et al., 2009), and blocking of SERT (all the study drugs have such an effect).

The study confirmed VOR's anti-depressant effect (reduction of immobility time) when administered repeatedly to both the NSC and PS groups, which corroborates the results of other authors (Pehrson et al., 2013; Katona and Katona, 2014). Pehrson et al. (2013) have shown that VOR's anti-depressant effect depended directly on the modulation of serotonin, dopamine and noradrenaline levels, particularly in the prefrontal cortex and hippocampus regions where a reduced number of nerve cells and impaired neurogenesis are observed. Pehrson et al. (2013) also found that deficiency of the monoamines mentioned above may reduce stress resistance and this may, consequently, lead to depression. This conclusion, thus confirms the potential effectiveness of prenatal stress as a contributor to depression in the animal model (Van den Hove, 2005). Katona and Katona (2014) have also shown that VOR, in addition to affecting neurotransmitters, also normalizes the GABA-ergic and glutaminergic systems. VOR's anti-depressant effect has also been confirmed in clinical studies by Baldwin et al. (2012) (using 2.5 mg, 5 mg, and 10 mg doses). Meanwhile, Boulenger et al. (2014) observed that the depression recurrence rate in patients receiving VOR was as low as 13%. It is believed that the anti-depressant effect of the drug arises largely from inhibition of 5-HT_{1A} autoreceptors and stimulation of 5-HT_{1A} heteroreceptors, and from blockade of SERT (Frank, 2008). The anti-depressant effect of VOR is also closely associated with 5-HT_3 receptor activity, which is influenced by regulation of sodium, potassium and calcium ion channels. When serotonin binds the 5-HT_3 receptor, the channel opens and the cell is depolarised (Artigas 2013). This paper also analyzed whether VOR modified animal locomotor activity. The results showed reduced mobility in animals receiving VOR upon chronic treatment, both in the NSC and PS groups, which may be a sign of sedation due to the drug's agonistic effect at 5-HT_{1A} autoreceptors situated in raphe nuclei and amygdaloid body. The drug's profile also includes anxiolytic efficacy (the drug facilitates falling asleep), which may be related to the observed reduction in locomotor activity. Another important mechanism of action of VOR is its partial agonism to 5-HT_{1B} receptors situated in the frontal lobe which - if blocked - reduce DA synthesis and release, thus reducing stimulation and mobility of the animals (Pytliak et al., 2011). The same receptor in the striatum, meanwhile, acts as an autoreceptor (presynaptic receptor) which may, in turn, contribute to inhibition of 5HT release and reduction of glutaminergic transmission resulting in the reduced post-synaptic potential of nerve cells (Pytliak et al., 2011). According to Feuerstein et al. (1996), the sedative effect observed upon VOR administration may also stem from its affinity to 5-HT_{1D} receptors which are responsible for proper tension of the smooth muscle of blood vessels. Our tests confirm the anxiolytic effect of VOR when administered once and repeatedly (after 7, 14, and 21 days) to both the NSC and PS groups. The results are in line with a study by Mørk et al. (2012) that demonstrated VOR administered subcutaneously at doses of 3.9 and 7.9 mg/kg had an anxiolytic effect on rats in an animal model of depression. The author showed that the anxiolytic effect resulted from the drug's partial agonism to 5-HT_{1B} receptor, increasing serotonin levels in the prefrontal cortex. Experiments on VOR were also conducted by Guilloux et al. (2013) who showed that the anxiolytic effect following a repeated administration of the drug is closely related to neurogenesis in the hippocampus as a result of pharmacological stimulation of 5-HT_{1A} receptor. The author further pointed out that the medicinal effect following VOR administration was evident even with low SERT binding, thus suggesting the presence of an additional mechanism inhibiting serotonin reuptake. In this context, the author (Guilloux et al., 2013) put forward 5-HT_3 ionotropic receptor as the additional actor, of which VOR is an antagonist. Blockade of this receptor following VOR administration may be responsible for the anxiolytic effect observed in our study. Pytka et al. (2015), on the other hand, described a significant role for 5-HT_7 receptor in the mechanism of action of the anxiolytic effect, and concluded that VOR's anxiolytic

effect results directly from the drug's antagonism of this receptor. A high density of 5-HT_7 receptors is observed primarily in the hippocampal area (Hedlund and Sutcliffe, 2004).

In turn, FLU reduced immobility time (an anti-depressant effect) in both the NSC and PS groups. These results are in alignment with a study by du Jardin et al. (2016) who demonstrated that FLU had an anti-depressant effect in Flinders Sensitive Line rats, a genetic model of depression. Moreover, Malberg and Duman (2003) demonstrated that inhibition of hippocampal cell proliferation, as a result of a stress factor (electrocution), reversed upon administration of FLU. An analysis of FLU's mechanism of action resulted in the conclusion that its anti-depressant effect was primarily a result of blockade of 5-HT_{2A} and 5-HT_{2C} receptors (Artigas, 2013) regulating mood, motor behavior and appetite (Millan 2005). These receptors have a post-synaptic location and are most dense in new cortex (receptor 5-HT_{2A}) (Burnet et al., 1995) as well as in black matter, cerebellum, and hippocampus (receptor 5-HT_{2C}) (Abramowski et al., 1995). The blockade of 5-HT_{2A} receptors increased serotoninergic transmission at 5-HT_{1A} receptors, especially in the limbic region (Santana et al., 2004) while the blockade of 5-HT_{2C} receptors, situated primarily around GABA-ergic neurons, may result in serotonin level reduction around raphe nuclei—a structure with 5-HT_{1A} autoreceptors whose primary function is regulation of serotonin levels in synaptic clefts (Serrats et al., 2005). Our analyses showed a reduction of locomotor activity in the NSC and PS groups upon chronic treatment with FLU. Regarding FLU, the achieved effect may be a direct result of inhibition of 5-HT_{2A} and 5-HT_{2C} receptors in the frontal lobe, primarily around the motor cortex responsible for motor behavior (Graziano et al., 2005). It was shown that FLU administered once and repeatedly (i.e., at 1, 7, 14 and 21 days) had an anxiolytic effect on both NSC and PS rats. The anxiolytic effect upon administration of FLU at a dose of 5 mg/kg was confirmed in studies by Nowakowska et al. (1996) who showed that, in addition to an anxiolytic and antidepressant effect, FLU also effectively improved memory. Drapier et al. (2007), meanwhile, found that FLU administered once at a dose of 5 mg/kg and 10 mg/kg was ineffective at reducing anxiety. Importantly, their results showed increased anxiety upon a single administration of FLU, which in this case may be related to 5-HT_{2C} receptor blockade (Drapier et al., 2007). Studies by Nash et al. (2008), showed that removal of the 5-HT_{1A} receptor from prefrontal cortex and limbic system of mice significantly increased anxiety in the animals, suggesting that the receptor is involved in the mechanism of anxiety generation and reduction.

DAP is an SSRI originally developed for treatment of depression and eventually approved for use in treatment of premature ejaculation in men. DAP's mechanism of action is based on inhibition of SERT and successive enhancement of 5-HT's effect on pre- and post-synaptic receptors of the sympathetic system (Jhanjee et al., 2011). Administration of this drug increases serotonin level in the synaptic cleft which re-stimulates these receptors, thus reducing libido and delaying ejaculation measured on the basis of prolonged intravaginal ejaculation latency time (Kendrici et al., 2007). In chemical terms, DAP is an SSRI; thus, it was of interest to investigate whether it also had an anti-depressant effect despite having a different therapeutic indication. DAP was shown to reduce immobility time (anti-depressant effect) in NSC rats upon chronic treatment and in PS rats upon either single or repeated administrations. As DAP is not used in the treatment of depression, there are no references regarding clinical aspects of the drug's use for the indication of depression or on its use in animal models. Nevertheless, such studies certainly appear necessary because of potential (negative) interactions upon the administration of DAP in the presence of other SERT-blocking agents. The study has also shown that DAP reduced the locomotor activity of animals in both NSC and PS groups, which is similar to the results obtained for administration of VOR and FLU—another SSRI drug. The result that a single administration of DAP resulted in increased locomotor activity in PS animals merits attention. No anxiolytic effect for DAP was observed in NSC rats, while administration of the drug to PS rats induced an anxiolytic effect upon single and repeated administrations (day 7, 14, 21). One of the mechanisms that might be responsible for DAP's anxiolytic effect in this group of animals is SERT inhibition which, consequently, may lead to increased serotonin levels in the synaptic cleft and anxiolytic effect.

Interestingly, combined administration of VOR+FLU to NSC animals was effective only upon single administration, while in the PS group this effect was observed for both single and chronic treatment. Both VOR and FLU in monotherapy reduced immobility time (an anti-depressant effect) only after 14 days, while the combination of these drugs was effective upon a single administration. Bhuvaneswari et al. (2015), studying the combined administration of SSRI drugs and aripiprazole, have shown that immobility time of animals upon combined administration of drugs was much shorter than for monotherapy. However, in combined therapy, attention must be paid to possible drug interactions. This applies specifically to using SSRIs which, combined with other drugs of the same group, may lead to serotonin syndrome, i.e., an excessive increase of 5-HT levels in the brain (Frank, 2008). In this context, the hypothetic possibility of an inadvertent combination of DAP with other SSRI agents by a patient seems a particularly relevant issue. It is highly

probable that a man suffering from premature ejaculation might receive a prescription for DAP from his sexual therapist or urologist and—as the same man might be undergoing treatment for depression due to "sexual dysfunction"—a SERT-blocking drug from his psychiatrist or general practitioner; this could potentially lead to the serotonin syndrome referred to above. This hypothesis may be partially confirmed by the results from our analysis of locomotor activity in animals in both NSC and PS animals showing that combined administration of VOR+FLU and DAP+FLU caused immediate sedation (upon a single administration of the drugs) that persisted during subsequent measurements. This study also tested the anxiolytic efficacy of combined therapy in NSC and PS rats. The results from the NSC group showed that only combined therapy with DAP+FLU had an anxiolytic effect, and only upon repeated administration (after 14 and 21 days). Studies by Londborg et al. (2000) and Barowsky and Schwartz (2006), as well as the results of our paper, show that the probable mechanism underlying the anxiolytic effect of DAP and FLU involves potentialization of serotonin reuptake by SERT (strong antagonism of the two drugs) which consequently causes 5-HT levels to increase in synaptic clefts, primarily within the prefrontal cortex, which is of fundamental importance in preventing depressive and anxiety-related disorders (Kamińska et al., 2013). It is particularly interesting that there is no anxiolytic effect (additive synergy) observed upon combined therapy with VOR and FLU in NSC rats, while the two drugs administered separately to non-stressed animals had a clear anxiolytic effect. Moreover, an anxiolytic effect of both separate and combined therapy with VOR and FLU was observed in the prenatally stressed group. Serotonin syndrome might be responsible for this result. Frank et al., (2008) noted that the many symptoms of serotonin syndrome may include increasing anxiety at lower levels of serotonin syndrome and suggested that it might be 5-HT_{1A} that is responsible for reducing anxiety symptoms. According to the Summary of Product Characteristics, VOR has an agonist effect on 5-HT_{1A} receptors, while experiments by Subhash et al. (2000) confirmed that FLU had this effect. According to a paper by Garcia-Garcia et al. (2014), there are two types of 5-HT_{1A} receptors—presynaptically located autoreceptors and postsynaptically located heteroreceptors. The author noted that an increase in the number of autoreceptors with a simultaneous decrease in the number of heteroreceptors contributes to emergence of symptoms of depression and anxiety. The autoreceptors' function is to reuptake serotonin from the synaptic cleft, while heteroreceptors located in the prefrontal cortex, the hippocampus, and the amygdala are responsible for mood and anxiety regulation (Garcia-Garcia et al., 2014). Therefore, these considerations lead to a conclusion that the absence of anxiolytic effects upon combined therapy with VOR+FLU may result from potentialization of 5-HT_{1A} autoreceptors, which then reuptake increased quantities of 5-HT from the synaptic cleft and may, in turn, reduce stimulation of 5-HT_{1A} heteroreceptors responsible for appearance of the anxiolytic effect. In the PS group, meanwhile, an anxiolytic effect was observed following combined therapy with VOR+FLU (single and repeated administration for 7, 14, and 21 days), and combined therapy with DAP+FLU (repeated administration—day 14 and 21). An enhanced anxiolytic effect following combined therapy with VOR+FLU in the group of prenatally stressed animals and absence of this effect in the non-stressed group may be related to neuroanatomical lesions within the hippocampus and prefrontal cortex (Ratajczak et al., 2013) caused by prenatal stress. These lesions may include lowered quantities of 5-HT_{1A} autoreceptors located on presynaptic membranes, thus leading to increased quantities of serotonin in the clefts and enhanced stimulation of 5-HT_{1A} (and other types of) receptors located postsynaptically. In other words, prenatal stress, by degenerating brain structures and affecting the quantity and density of reception, may increase their sensitivity by way of up-regulation. This hypothesis would, therefore, explain why combined therapy with VOR+FLU had an anxiolytic effect in the PS group while in it did not in NSC group, and underlines the importance of environmental/external factors which may affect the therapy's efficacy.

CONCLUSION

Moreover, analysis of the pathomechanism of depression and the mechanisms of action of individual drugs allows for the assumption that the combined administration of the tested drugs (particularly DAP) may be effective in the treatment of depressive and anxiety disorders, although possible negative interactions between treatment drugs must always be assessed for. Lastly, the results also showed that the antidepressant efficacy of VOR and FLU are similar.

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