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Comparison of the effects of 1MeTIQ and olanzapine on performance in the elevated plus maze test and monoamine metabolism in the brain after ketamine treatment

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ABSTRACT

Anxiety is a common symptom of schizophrenia. Ketamine, which acts as a noncompetitive antagonist of glutamatergic NMDA receptors by binding to the phencyclidine site, may induce schizophrenia-like symptoms and promote anxiogenic-like behaviour. The symptoms of anxiety in rodents can be measured by the elevated plus maze (EPM) test. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ), as a neuroprotective and antiaddictive substance, produces pharmacological effects by influencing monoaminergic and glutamatergic activity, as previously demonstrated by us. The aim of the present study was to investigate the anxiolytic-like potential of 1MeTIQ after the administration of ketamine. These results were compared to the effects of olanzapine, an antipsychotic drug commonly used in the treatment of schizophrenia. We conducted the EPM test, during which the percentage of time spent in and the number of entries into the open arms were measured. In addition, locomotor activity was measured. Furthermore, we conducted biochemical analyses to verify changes in the levels of neurotransmitters and their metabolites in selected rat brain structures. Behavioural analyses showed that 1MeTIQ, similar to olanzapine, completely inhibited ketamine-induced anxiogenic effects in the EPM test. On the other hand, neurochemical data indicated that 1MeTIQ, as a reversible inhibitor of MAO, significantly blocked the dopamine MAO-dependent oxidation pathway, whereas olanzapine significantly increased the activity of this pathway. The results above suggest that the anxiolytic-like properties of 1MeTIQ are connected to its influence on the catabolism of dopamine, the elevation of serotonin concentrations and the reduction in the levels of noradrenaline.

1. Introduction

Anxiety is one of the common symptoms of schizophrenia; it occurs in 38.3% of patients with this disorder and impacts disease outcomes (Braga et al., 2013). Patients with anxiety disorders suffer from physical and emotional discomfort and often need pharmacological treatment. The majority of anxiolytic drugs (benzodiazepines) affect the GABAergic system, but there is a need to develop other effective agents that affect serotoninergic or endocannabinoid transmission (Griebel and Holmes, 2013). Noradrenaline (NA) is also thought to modulate anxiety/fear behaviour (Tanaka et al., 2000). A study in mice showed that increased levels of NA promote anxiogenic behaviour and that the inhibition of noradrenergic neurons suppresses this process (McCall et al., 2015). The brain noradrenergic system is activated by acute stress, and at the same time, this stress produces anxiety-like behaviour that is associated with a reduction in open arm exploration in the elevated plus-maze (EPM) test (Morilak et al., 2005; Ehlers and Todd, 2017). Furthermore, experimental studies and human research have demonstrated that the application of alpha1- and beta-adrenergic antagonists has been further shown to reduce anxiety (McCall et al., 2015; Ehlers and Todd, 2017).

Ketamine, as an antagonist of NMDA receptors, may induce schizophrenia-like symptoms (Frohlich and Van Horn, 2014), and some studies have shown that ketamine promotes anxiogenic behaviour in animals (Silvestre et al., 1997; Babar et al., 2001; da Silva et al., 2010) and in healthy humans (Krystal et al., 1999). According to some studies,

Abbreviations: COMT, catechol-*O*-methyltransferase; DOPAC, 3,4-dihydroxyphenylacetic acid; EPM, elevated plus maze; GABA, gamma-aminobutyric acid; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; MAO, monoamine oxidase; 1MeTIQ, 1-methyl-1,2,3,4-tetrahydroisoquinoline; MPP^+, 1-methyl-4-phenylpyridinium ion; 3-MT, 3-methoxytyramine; NM, normetanephrine; NMDA, *N*-methyl-D-aspartate; ROS, reactive oxygen species; SERT, serotonin transporter

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ketamine is able to block the reuptake of catecholamines by diminishing COMT activity (Koehntop et al., 1977). Therefore, ketamine administration has been used to produce an animal model of schizophrenia. The EPM is an apparatus used to measure symptoms of anxiety in rodents (Costa et al., 2014). In particular, the parameters that are measured, such as the number of entries into and the time spent in the open arms, measure anxiety-like behaviour. Additional parameters that can be measured in the EPM are occurrences of grooming and rearing and the distance travelled.

Olanzapine is a second-generation (atypical) antipsychotic drug widely used in the treatment of schizophrenia (Arakawa et al., 2010; Komossa et al., 2010; Mendonca Jùnior et al., 2015). The anxiolytic effect of olanzapine in humans has been reported (Suppes et al., 2017; Kulhan et al., 2018), and it has also been indicated that olanzapine may reduce episodes of depression in bipolar disorder and may contribute to preventing episodes of mania. Nevertheless, olanzapine may induce many side effects in humans, such as weight gain or sedation (Pan et al., 2014). Similar anxiolytic properties have been described in animal studies (Ratajczak et al., 2016a; Ratajczak et al., 2016b). Olanzapine binds to two primary therapeutic receptor targets, one in the serotoninergic system (the 5HT2A receptor) and one in the dopaminergic system (the D_2 receptor) (Lian et al., 2013). Moreover, olanzapine also shows affinity for adrenergic alpha1, histaminergic H1, and muscarinic M1–M5 receptors and thus may be considered a multi-acting receptor-targeted drug (Nagai and Watanabe, 2013). The functional blockade of a variety of receptors contributes to the broad spectrum of the therapeutic properties of olanzapine.

1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous amine present naturally in the mammalian brain at low concentrations (Antkiewicz-Michaluk et al., 2014). We have previously demonstrated that 1MeTIQ is a dopamine receptor partial agonist and a reversible MAO inhibitor that has been shown to have neuroprotective (Antkiewicz-Michaluk et al., 2006, 2007; Patsenka and Antkiewicz-Michaluk, 2004; Wąsik et al., 2016) and antiaddictive (Wąsik et al., 2010) properties and to increase dopamine levels, which suggests that it may be a helpful agent in the treatment of Parkinson's disease (Wąsik et al., 2018). Our earlier results have shown that the neuroprotective mechanism of 1MeTIQ is closely related to its free radical scavenging properties and its inhibition of glutamate-induced excitotoxicity, as demonstrated in both in vitro and in vivo microdialysis studies (Antkiewicz-Michaluk et al., 2006; Kuszczyk et al., 2014). Further research has led to the conclusion that 1MeTIQ plays an important role as a protector of dopaminergic neurons due to its anti-oxidative properties and that the action of 1MeTIQ is blocked by neurotoxic substances used to cause parkinsonism in animal models such as MPTP and β-carbolines (Antkiewicz-Michaluk et al., 2014). Moreover, 1MeTIQ may be considered in the treatment of schizophrenia as a partial dopamine agonist and a neuroprotective compound.

The aim of the present study was to investigate the anxiolytic-like potential of 1MeTIQ after the administration of ketamine to induce anxiogenic behaviours. The results of 1MeTIQ treatment were compared to the effect of olanzapine, an antipsychotic drug with a proven mechanism of action that is commonly used in the treatment of schizophrenia. We used the EPM test, during which we measured the percentage of time spent in and the number of entries into the open arms. In addition, the locomotor activity of the rats was measured. Biochemical analysis was performed to verify changes in the levels of neurotransmitters and their metabolites in selected rat brain structures.

2. Materials and methods

2.1. Animals and treatments

All experiments were carried out on male Sprague-Dawley rats with an initial body weight of 225–250 g. The animals were kept in the standard polyacrylic cages (5 animals/cage). All animals had free access to standard laboratory food and tap water and were kept at room temperature (22 °C) under an artificial light/dark cycle (12/12 h, light on at 7:00). One of two doses of 1MeTIQ (25 or 50 mg/kg, intraperitoneally (i.p.)) or olanzapine (3 mg/kg i.p.) was administered once 20 min before the ketamine (10 mg/kg i.p.) injection. Control rats were treated with an appropriate vehicle (0.9% NaCl). The elevated plus maze (EPM) test was conducted 90 min after ketamine administration. All experimental groups consisted of 10 individuals. The doses of 1MeTIQ used in this experiment were based on our previous experience, while doses of olanzapine (Rogóż and Skuza, 2011) and ketamine were based on literature (Silvestre et al., 1997). Immediately after the behavioural experiments, the rats were killed by decapitation, and different structures of the brain were dissected out for later analysis. The experiments were carried out between 9.00 and 16.00 h.

All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health and received approval from the Bioethics Commission as compliant with Polish law.

2.2. Drugs

1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) was synthesized by the Department of Drug Chemistry, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland. The purity of the compound was verified by measurement of the melting point, and homogeneity was assessed on a chromatographic column. Ketamine (Biowet Puławy, Poland) in the form of a solution for injection (ketamine hydrochloride). Olanzapine (Sigma-Aldrich, USA) was suspended in a 1% aqueous solution of Tween 80; 1MeTIQ was dissolved in sterile 0.9% NaCl solution and injected in a volume of 1 ml/kg.

2.3. The EPM test

The apparatus, made of Plexiglas and elevated to the height of 50 cm, consisted of two open arms (40 × 12 cm) and two closed arms (40 × 12 × 20 cm) placed perpendicular to each other and extending from a central platform (12 × 12 cm). The experiments were conducted under a low-intensity light (30 lx). The test was initiated by placing a rat on the central platform of the maze, facing an open arm. Testing lasted for 5 min, and the number of open- and closed-arm entries was recorded using the Any-maze® tracking system. The percentage of open-arm entries (total number of entries / number of open-arm entries × 100) and the percent of time spent in the open arms served as the measure of anxiety. Additionally, rearing behaviour and the total number of entries (open- and closed-arms) were used as a measure of locomotor activity. The maze was thoroughly cleaned after each trial (Nikiforuk et al., 2011).

2.4. Ex vivo biochemical studies

Immediately after the behavioural experiments, the rats were killed by decapitation, and the frontal cortex, hippocampus, striatum and nucleus accumbens were dissected. The obtained tissue was frozen on solid CO_2 (−70 °C) and stored until used for biochemical assays. Dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and final metabolite, homovanillic acid (HVA); serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA); noradrenaline and its metabolite normetanephrine (NM) were assayed by means of high-performance liquid chromatography (HPLC) with electrochemical detection. The chromatograph (HP 1050; Hewlett-Packard, Golden, CO, USA) was equipped with C18 columns. The tissue samples were weighed and homogenized in ice-cold 0.1 M trichloroacetic acid containing 0.05 mM ascorbic acid. After centrifugation (10,000 × *g*, 5 min), the supernatants were filtered through RC 58 0.2-μm cellulose membranes (Bioanalytical Systems, West Lafayette, IN, USA). The mobile phase consisted of 0.05 M citrate-

phosphate buffer, pH 3.5, 0.1 mM EDTA, 1 mM sodium octyl sulfonate and 3.5% methanol. The flow rate was maintained at 1 ml/min. Dopamine, serotonin, noradrenaline and their metabolites were quantified by peak height comparisons with standards run on the day of analysis.

2.5. Statistical analysis

The results of behavioural tests were analysed by means of a one-way analysis of variance (ANOVA) followed, when appropriate, by Fisher's post-hoc tests. The data from the neurochemical studies were analysed by means of a one-way ANOVA followed, when appropriate, by Duncan's post-hoc tests. The results were considered statistically significant when $p < 0.05$.

3. Results

3.1. The behavioural EPM test

The statistical analyses of the behavioural results showed that the percentage of time spent in the open arms of the maze significantly differed among the eight groups ($F[7,61] = 3.07$, $p < 0.05$) (Fig. 1A). Fisher's post hoc tests indicated that the acute dose of ketamine significantly decreased ($p < 0.05$) the percentage of time spent in the open arms compared to the control (saline) group. 1MeTIQ administered at a low dose (25 mg/kg i.p.) with ketamine did not change this effect in the EPM test. However, 1MeTIQ given at the higher dose (50 mg/kg i.p.) as well as olanzapine (3 mg/kg i.p.) completely blocked the ketamine-induced effect (Fig. 1A).

One-way ANOVA indicated no significant effect of treatment on the percentage of open-arm entries ($F[7,68] = 2.04$, N.S.) (Fig. 1B).

In the same experiment, the statistical analysis revealed a significant effect on the rats' locomotor activity (the total number of entries) ($F[7,71] = 3.77$, $p < 0.01$) (Fig. 1C). The post hoc tests demonstrated that 1MeTIQ (50 mg/kg i.p) given combined with ketamine produced a significant decrease in locomotor activity ($p < 0.05$) compared to the control group. A similar effect was observed after olanzapine (3 mg/kg i.p.) administration ($p < 0.01$). Additionally, administration of olanzapine with ketamine also significantly reduced the total number of entries ($p < 0.05$) (Fig. 1C).

One-way ANOVA showed that rearings also significantly differed among the eight groups ($F[7,60] = 5.64$, $p < 0.01$) (Fig. 1D). Fisher's post hoc tests revealed that both 1MeTIQ (50 mg/kg i.p.) and olanzapine (3 mg/kg i.p.) given alone significantly reduced the number of rearings ($p < 0.05$). A similar effect was observed in the drug combination groups when 1MeTIQ and olanzapine were injected with ketamine ($p < 0.05$) (Fig. 1D).

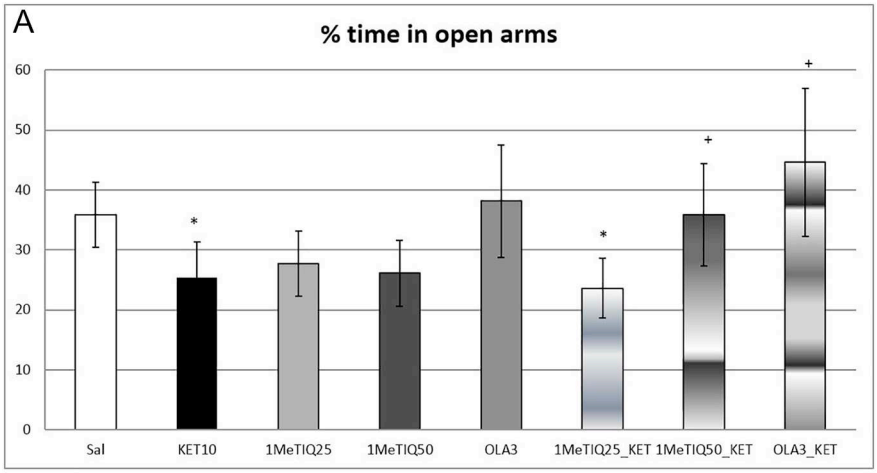
3.2. Biochemical studies

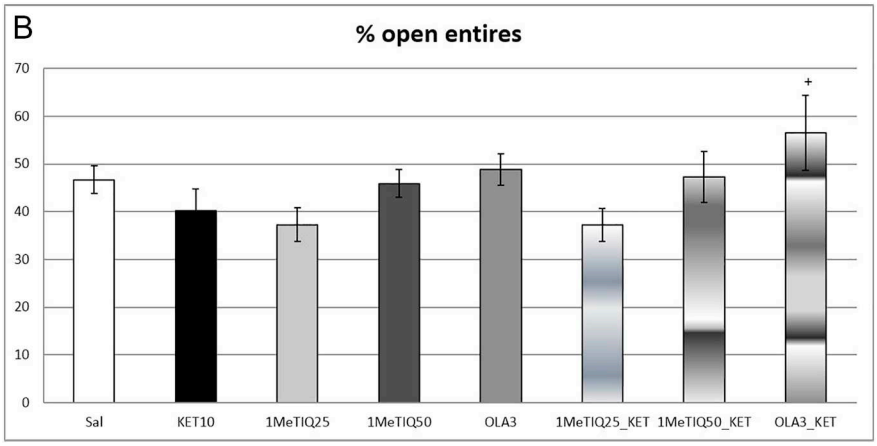
3.2.1. The impact of 1MeTIQ and olanzapine on ketamine-induced changes in dopamine (DA) and its metabolism in the rat brain

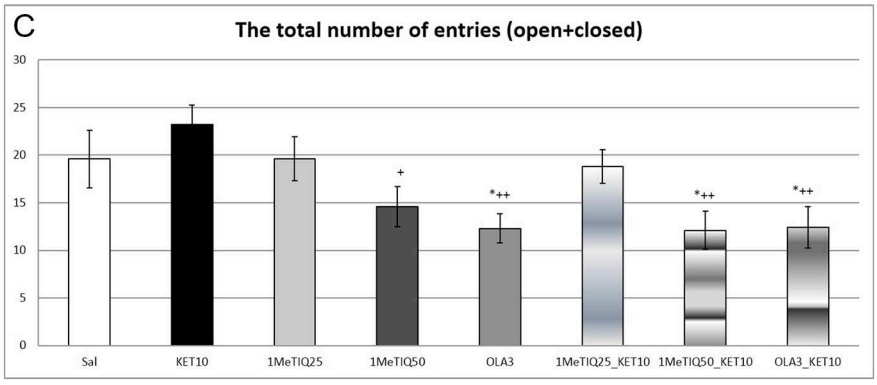
3.2.1.1. Frontal cortex (Fcx). One-way ANOVA demonstrated a significant effect of treatment ($F[7,40] = 3.30$, $p < 0.01$) on the levels of DA in the Fcx (Table 1). Duncan's post hoc tests demonstrated that the acute dose of ketamine did not change the concentration of DA, while 1MeTIQ (25 mg/kg) with ketamine and 1MeTIQ (50 mg/kg) given alone significantly increased levels of DA (60% and 45%, respectively; $p < 0.01$) in the Fcx. The statistical analysis indicated a significant effect of treatment ($F[7,40] = 39.6$, $p < 0.01$) on DOPAC concentrations in the Fcx (Table 1). Post hoc analysis showed no significant effect of ketamine. However, olanzapine given alone and with ketamine significantly elevated levels of DOPAC in the Fcx (~150%; $p < 0.01$) (Table 1). One-way ANOVA demonstrated a significant effect ($F[7,40] = 2.8$, $p < 0.05$) on the levels of 3-MT in the Fcx (Table 1). Duncan's post hoc test showed that the acute injection of ketamine did not change the concentration of 3-MT in the rat Fcx. However, 1MeTIQ (50 mg/kg) given alone and with ketamine significantly reduced concentrations of 3-MT in this structure (50% and 60%, respectively; $p < 0.05$). The strongest effect was observed in the group treated with olanzapine and ketamine (a reduction of ~70%). The statistical analysis indicated a significant effect of treatment ($F[7,40] = 67.8$, $p < 0.01$) on HVA concentrations in the Fcx (Table 1). Duncan's post hoc analysis demonstrated that ketamine did not change the concentration of HVA, while 1MeTIQ (50 mg/kg) given alone and with ketamine decreased levels of HVA (50% and 60%, respectively; $p < 0.01$). In contrast, olanzapine given alone and with ketamine strongly increased concentrations of HVA (~300%) (Table 1). One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 5.55$, $p < 0.01$) on dopamine reuptake measured by the index [3-MT] / [DA] × 100 in the Fcx (Table 1). Post hoc analysis showed that ketamine significantly decreased this index (~50%, $p < 0.01$). A similar effect was observed in both groups with 1MeTIQ administered alone (50% and 60%, respectively, $p < 0.01$). Moreover, olanzapine and both 1MeTIQ doses given together with ketamine significantly reduced the [3-MT]/[DA] index (~60%, $p < 0.01$) (Table 1). The statistical analysis demonstrated a significant effect of treatment ($F[7,40] = 37$, $p < 0.01$) on total dopamine metabolism measured by the index [HVA]/[DA]x100 in the Fcx (Table 1). Duncan's post hoc test showed that the acute dose of ketamine did not change this index. However, olanzapine administered alone and with ketamine strongly elevated the total dopamine metabolism (300% and 400%, respectively, $p < 0.01$) (Table 1). In contrast, 1MeTIQ (50 mg/kg) given together with ketamine decreased this index (~75%, $p < 0.05$) in the Fcx (Table 1).

3.2.1.2. Hippocampus. One-way ANOVA indicated a significant effect of treatment ($F[7,37] = 4.1$, $p < 0.01$) on DA concentrations in the hippocampus (Table 1). Duncan's post hoc test demonstrated that the acute dose of ketamine did not change the level of DA. In contrast, both doses of 1MeTIQ (25 and 50 mg/kg) with ketamine and 1MeTIQ (50 mg/kg) given alone significantly increased levels of DA (80%, 95% and 90%, respectively; $p < 0.01$) in the hippocampus (Table 1). The statistical analysis showed a significant effect of treatment ($F[7,39] = 10.65$, $p < 0.01$) on DOPAC concentrations in the hippocampus (Table 1). Post hoc analysis revealed no significant effect of ketamine. However, olanzapine given alone and with ketamine significantly elevated levels of DOPAC in the hippocampus (~100%; $p < 0.01$) (Table 1). One-way ANOVA demonstrated no significant effect of treatment on 3-MT ($F[7,32] = 1.02$, N.S.) and HVA ($F[7,40] = 2.06$, N.S.) concentrations in the hippocampus (Table 1). The statistical analysis showed no significant effect of treatment ($F[7,40] = 1.32$, N.S.) on dopamine reuptake measured by the index [3-MT]/[DA]x100 in the hippocampus (Table 1). The same analysis indicated a significant effect of treatment ($F[7,40] = 2.3$, $p < 0.05$) on total dopamine metabolism measured by the index [HVA]/[DA]x100 in the hippocampus (Table 1). Duncan's post hoc tests showed that olanzapine given with ketamine led to a significant increase (~100%, $p < 0.05$), while 1MeTIQ (50 mg/kg) administered with ketamine resulted in a substantial reduction (~90%, $p < 0.01$) in this measure (Table 1).

3.2.1.3. Striatum. The statistical analysis revealed a significant effect of treatment ($F[7,40] = 5.76$, $p < 0.01$) on DA concentrations in the striatum (Table 1). Duncan's post hoc tests demonstrated that the acute dose of ketamine did not change the level of DA. However, a higher dose of 1MeTIQ (50 mg/kg) given alone and the treatment of 1MeTIQ (25 mg/kg) with ketamine significantly elevated DA concentrations (20% and 30%, respectively). One-way ANOVA indicated a significant effect of treatment ($F[7,39] = 79.66$, $p < 0.01$) on DOPAC concentrations in the rat striatum (Table 1). The post hoc analyses showed that olanzapine given alone and with ketamine significantly







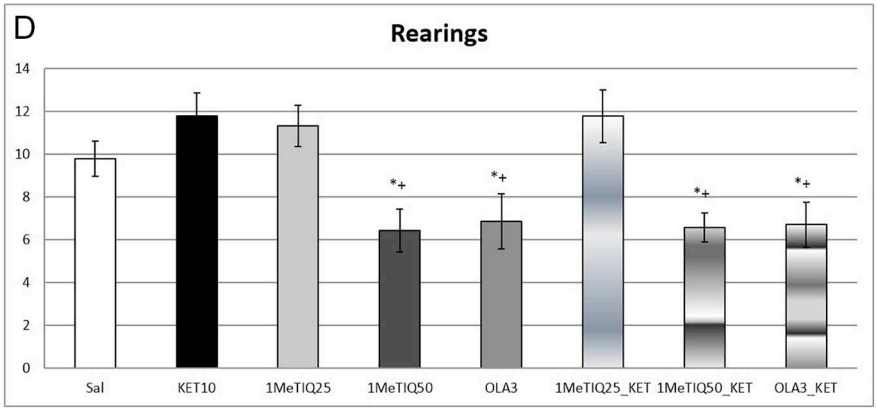


Fig. 1. The effect of 1MeTIQ and olanzapine (OLA) on ketamine-induced changes in the elevated plus maze (EPM) test. The rats received a single injection of saline (control), ketamine (KET) (10 mg/kg i.p.), 1MeTIQ (either 25 or 50 mg/kg i.p.) or OLA (3 mg/kg i.p.). In the combination groups, 1MeTIQ or OLA was given 20 min before the KET (10 mg/kg i.p.) injection. The behavioural test (EPM) was conducted 90 min after KET injection. The rats were placed into the EPM apparatus for 5 min. During this time, different types of behaviour were measured: % time spent in open arms (Fig. 1A), % open arm entries (Fig. 1B), the total number of entries (Fig. 1C) and a number of rearings (Fig. 1D). The data are means ± S.E.M., and the number of animals was n = 9–10; *$p < 0.05$ and **$p < 0.01$ indicate a difference from the control group (SAL) and $^{+}p < 0.05$ and $^{++}p < 0.01$ indicate a difference from the KET group with Fisher's test.

Table 1
The impact of 1MeTIQ, olanzapine (OLA) and ketamine (KET) on dopamine (DA) levels and its metabolism in the rat brain.

Frontal cortex

Treatment	DA (ng/g t)	DOPAC (ng/g t)	3-MT (ng/g t)	HVA (ng/g t)	3-MT/DA	HVA/DA
Saline	422 ± 35	132 ± 10	7,5 ± 0,8	181 ± 10	1,85 ± 0,26	44,6 ± 4,9
KET_10	493 ± 29	139 ± 8	4,5 ± 1,3	194 ± 12	0,87 ± 0,22**	40 ± 3,6
OLA_3	463 ± 48	334 ± 24**++	6 ± 0,3	575 ± 23**++	1,38 ± 0,11	130 ± 11,7**++
MeTIQ_25	568 ± 65	93 ± 8	6 ± 1,9	169 ± 16	0,97 ± 0,22**	31 ± 4
MeTIQ_50	614 ± 51*	80 ± 5+	3,7 ± 0,8*	99 ± 31*+	0,61 ± 0,14**	15 ± 3,8
OLA3_KET	382 ± 49	322 ± 33**++	2,1 ± 0,4**	583 ± 48**++	0,61 ± 0,15**	165 ± 22**++
MeTIQ25_KET	672 ± 64**+	115 ± 6	5,5 ± 1,4	172 ± 22	0,87 ± 0,22**	27 ± 3,9
MeTIQ50_KET	588 ± 86	87 ± 13	2,8 ± 0,7*	59 ± 12**++	0,53 ± 0,13**	10,6 ± 2*
F	F(7/40) = 3,30 p < 0,01	F(7/40) = 39,59 p < 0,01	F(7/40) = 2,8 p < 0,05	F(7/40) = 67,83 p < 0,01	F(7/40) = 5,55 p < 0,01	F(7/40) = 37 p < 0,01

Hippocampus

Treatment	DA (ng/g t)	DOPAC (ng/g t)	3-MT (ng/g t)	HVA (ng/g t)	3-MT/DA	HVA/DA
Saline	51 ± 9	18 ± 3	44 ± 15	25 ± 8	101 ± 34	57 ± 21
KET_10	56 ± 9	24 ± 4	58 ± 6	33 ± 10	134 ± 36	76 ± 32
OLA_3	65 ± 6	37,5 ± 3**++	51,7 ± 12	44 ± 16	85 ± 22	67 ± 26
MeTIQ_25	65 ± 4	19,7 ± 2	66 ± 15	25 ± 9	100 ± 20	40 ± 14
MeTIQ_50	92 ± 3**+	19,5 ± 2	73 ± 6	12 ± 7	80 ± 5	6 ± 5
OLA3_KET	65 ± 8	40 ± 3**++	74 ± 16	61 ± 22	133 ± 43	113 ± 45*
MeTIQ25_KET	86 ± 11*+	19,5 ± 2	56,5 ± 9	17 ± 10	70 ± 7	22 ± 14
MeTIQ50_KET	97 ± 11**++	19,8 ± 1	46 ± 3	7 ± 6	49 ± 4	7 ± 6*+
F	F(7/37) = 4,1 p < 0,01	F(7/39) = 10,65 p < 0,01	F(7/32) = 1,02 N.S.	F(7/40) = 2,06 N.S.	F(7/40) = 1,32 N.S.	F(7/40) = 2,3 P < 0,05

Striatum

Treatment	DA (ng/g t)	DOPAC (ng/g t)	3-MT (ng/g t)	HVA (ng/g t)	3-MT/DA	HVA/DA
Saline	10,472 ± 181	1337 ± 58	306 ± 42	1136 ± 95	2,9 ± 0,4	11 ± 0,9
KET_10	9904 ± 325	1328 ± 72	291 ± 18	1073 ± 50	2,9 ± 0,2	11 ± 0,3
OLA_3	10,704 ± 298	4053 ± 176**	280 ± 26	4022 ± 125**	2,6 ± 0,2	38 ± 1,5**++
MeTIQ_25	10,972 ± 682	1413 ± 47	657 ± 20**+	1191 ± 61	6,1 ± 0,3**++	11 ± 0,6
MeTIQ_50	11,699 ± 345*	767 ± 37*	803 ± 43**	1011 ± 67	6,9 ± 0,5**++	9 ± 0,5
OLA3_KET	10,544 ± 187	4619 ± 289**++	275 ± 33	4384 ± 250**++	2,6 ± 0,3	41 ± 2,1**++
MeTIQ25_KET	12,884 ± 390**++	1002 ± 40	443 ± 36*	1222 ± 70	3,4 ± 0,3	9 ± 0,5
MeTIQ50_KET	10,280 ± 500	1757 ± 345	1021 ± 90**++	1190 ± 114	10 ± 0,8**++	11 ± 0,9
F	$F_{(7/40)}$ = 5,76 p < 0,01	$F_{(7/39)}$ = 79,66 p < 0,01	$F_{(7/40)}$ = 42,07 p < 0,01	$F_{(7/40)}$ = 140,02 p < 0,01	$F_{(7/40)}$ = 39,6 p < 0,01	$F_{(7/40)}$ = 158 p < 0,01

Nucleus accumbens

Treatment	DA (ng/g t)	DOPAC (ng/g t)	3-MT (ng/g t)	HVA (ng/g t)	3-MT/DA	HVA/DA
Saline	9608 ± 242	2250 ± 110	176 ± 30	1124 ± 71	1,8 ± 0,3	12 ± 0,7
KET_10	8648 ± 418	1775 ± 109	160 ± 18	1099 ± 52	1,9 ± 0,2	13 ± 0,4
OLA_3	9614 ± 256	4526 ± 248**	157 ± 23	3866 ± 203**	1,6 ± 0,2	40 ± 2,2**++
MeTIQ_25	9539 ± 1102	1163 ± 130**	210 ± 31	965 ± 128	2,7 ± 0,8	10 ± 0,5
MeTIQ_50	10,124 ± 503	1090 ± 63**	424 ± 22**	984 ± 68	4,2 ± 0,3**++	10 ± 0,7
OLA3_KET	9017 ± 395	5520 ± 522**++	160 ± 26	4372 ± 371**++	1,8 ± 0,3	49 ± 4**++
MeTIQ25_KET	10,815 ± 443+	1391 ± 124*	211 ± 40	1254 ± 122	1,9 ± 0,3	11 ± 0,9
MeTIQ50_KET	8193 ± 862	1015 ± 61**+	373 ± 49**++	831 ± 88	4,5 ± 0,3**++	10 ± 0,6
F	$F_{(7/40)}$ = 1,91 NS	$F_{(7/40)}$ = 59,77 p < 0,01	$F_{(7/40)}$ = 11,16 p < 0,01	$F_{(7/40)}$ = 71,38 p < 0,01	$F_{(7/40)}$ = 7,31 p < 0,01	$F_{(7/40)}$ = 93,4 p < 0,01

The rats received a single injection of saline (control), ketamine (KET) (10 mg/kg i.p.), 1MeTIQ (either 25 or 50 mg/kg i.p.) or olanzapine (OLA) (3 mg/kg i.p.). In the combination group, 1MeTIQ or OLA was given 20 min before the KET (10 mg/kg i.p.) injection. Data were analysed by one-way ANOVA followed by Duncan's post hoc tests. The data are means ± S.E.M. and the number of animals was n = 6; *p < 0.05 and **p < 0.01 indicate a difference from the control group (SAL); +P < 0.05 and ++ P < 0.01 indicate a difference from the KET group.

elevated levels of DOPAC in the striatum (~350%; p < 0.01) (Table 1). In contrast, 1MeTIQ (50 mg/kg) produced a significant reduction in the DOPAC concentration (~45%). The statistical analysis demonstrated a significant effect of treatment (F[7,40] = 42.07, p < 0.01) on 3-MT concentrations in the striatum (Table 1). Duncan's post hoc tests showed that both doses of 1MeTIQ strongly increased levels of 3-MT (100% and 180%, respectively). This effect was even stronger in the drug combination group, where 1MeTIQ (50 mg/kg) was administered

together with ketamine (an increase of ~300%). The statistical analysis indicated a significant effect of treatment ($F[7,40] = 140.02$, $p < 0.01$) on HVA concentrations in the striatum (Table 1). The post hoc tests revealed that both olanzapine groups, alone and with ketamine, significantly elevated levels of HVA (~400%). One-way ANOVA demonstrated a significant effect of treatment ($F[7,40] = 39.6$, $p < 0.01$) on dopamine reuptake measured by the index [3-MT]/[DA]x100 in the striatum (Table 1). Duncan's post hoc tests showed that both doses of 1MeTIQ increased this index (~100%, $p < 0.01$). This effect was also observed when 1MeTIQ (50 mg/kg) was administered with ketamine (~300%, $p < 0.01$) (Table 1). The statistical analysis indicated a significant effect of treatment ($F[7,40] = 158$, $p < 0.01$) on total dopamine metabolism measured by the index [HVA] / [DA] × 100 in the striatum (Table 1). The post hoc tests revealed that both olanzapine groups, alone and with ketamine, significantly elevated this index (~400%, $p < 0.01$) (Table 1).

3.2.1.4. Nucleus accumbens. One-way ANOVA demonstrated no significant effect of treatment on DA concentrations ($F[7,40] = 1.91$, N.S.) in the nucleus accumbens (Table 1). The same analysis indicated a significant effect of treatment ($F[7,40] = 59.77$, $p < 0.01$) on DOPAC levels in the nucleus accumbens (Table 1). Duncan's post hoc tests showed that both doses of 1MeTIQ given alone and with ketamine significantly reduced DOPAC concentrations (~55%). In contrast, olanzapine given alone and with ketamine increased levels of DOPAC (100% and 150%, respectively). The statistical analysis showed a significant effect of treatment ($F[7,40] = 11.16$, $p < 0.01$) on 3-MT concentrations (Table 1). The post hoc analyses showed that both 1MeTIQ given alone and with ketamine significantly elevated levels of 3-MT (150% and 120%, respectively). One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 71.38$, $p < 0.01$) on HVA concentrations in the nucleus accumbens (Table 1). Duncan's post hoc tests showed that olanzapine given both alone and with ketamine significantly elevated levels of HVA (330% and 400%, respectively). One-way ANOVA demonstrated a significant effect of treatment ($F[7,40] = 7.31$, $p < 0.01$) on dopamine reuptake measured by the index [3-MT]/[DA]x100 in the nucleus accumbens (Table 1). Duncan's post hoc tests showed that 1MeTIQ (50 mg/kg) given alone and with ketamine increased this index (~130%, $p < 0.01$). (Table 1). The statistical analysis indicated a significant effect of treatment ($F[7,40] = 93.4$, $p < 0.01$) on total dopamine metabolism as measured by the index [HVA]/[DA]x100 in the nucleus accumbens (Table 1). The post hoc tests revealed that both olanzapine groups, alone and with ketamine, significantly elevated this index (300% and 400%, respectively, $p < 0.01$) (Table 1).

3.2.2. The impact of 1MeTIQ and olanzapine on ketamine-induced changes in serotonin (5-HT) and noradrenaline (NA) and their metabolism in the rat brain

3.2.2.1. Frontal cortex (Fcx). One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 14.14$, $p < 0.01$) on 5-HT concentrations in the Fcx (Table 2). Duncan's post hoc tests showed that 1MeTIQ (25 mg/kg) given alone and with ketamine significantly increased 5-HT concentrations (~35%; $p < 0.01$). A similar effect was observed after a higher dose of 1MeTIQ (50 mg/kg). In contrast, olanzapine with ketamine significantly decreased levels of 5-HT (~25%; $p < 0.05$) (Table 2). The same analysis indicated a significant effect of treatment ($F[7,40] = 5.19$, $p < 0.01$) on 5-HIAA concentrations in the Fcx (Table 2). The post hoc test demonstrated that 1MeTIQ (50 mg/kg) reduced the level of 5-HIAA (~25%; $p < 0.05$) in the Fcx. One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 19.4$, $p < 0.01$) on the rate of serotonin metabolism measured by the index [5-HIAA]/[5-HT]x100 in the Fcx (Table 2). Duncan's post hoc test showed that 1MeTIQ (25 or 50 mg/kg) given alone and with ketamine significantly reduced this index (~40%, $p < 0.01$). At the same time, olanzapine with ketamine elevated the rate of serotonin metabolism (~20%, $p < 0.01$) in the Fcx (Table 2).

One-way ANOVA showed a significant effect of treatment ($F[7,40] = 3.91$, $p < 0.01$) on NA concentrations in the Fcx (Table 2). The post hoc analyses indicated that olanzapine alone and with ketamine, significantly reduced NA concentrations (~20%; $p < 0.05$) in the Fcx. The statistical analysis revealed a significant effect of treatment ($F[7,40] = 15.7$, $p < 0.01$) on NM concentrations in the Fcx (Table 2). Duncan's post hoc tests showed that 1MeTIQ (25 or 50 mg/kg) given alone significantly increased levels of NM (25%, $p < 0.05$ and 100%, $p < 0.01$; respectively). Moreover, 1MeTIQ (50 mg/kg) with ketamine also produced an elevation in NM concentration (~95%; $p < 0.01$) in the Fcx (Table 2). One-way ANOVA showed a significant effect of treatment ($F[7,40] = 7.9$, $p < 0.01$) on the rate of noradrenaline metabolism measured by the index [NM] / [NA] × 100 in the Fcx (Table 2). The post hoc analyses indicated that 1MeTIQ (50 mg/kg) alone and with ketamine, significantly increased this index (~100%, $p < 0.01$).

3.2.2.2. Hippocampus. One-way ANOVA indicated a significant effect of treatment ($F[7,36] = 2.39$, $p < 0.05$) on 5-HT concentrations in the hippocampus (Table 2). Post hoc analysis showed that an acute dose of ketamine significantly decreased the 5-HT concentration in the hippocampus (~45%; $p < 0.05$). A similar effect was observed in the olanzapine group and when either olanzapine or 1MeTIQ (50 mg/kg) was administered with ketamine (~45%; $p < 0.05$). The statistical analysis demonstrated a significant effect of treatment ($F[7,40] = 6.71$, $p < 0.01$) on 5-HIAA concentrations in the hippocampus (Table 2). Duncan's post hoc tests showed that 1MeTIQ (50 mg/kg) given alone and with ketamine significantly reduced levels of 5-HIAA (~35%; $p < 0.01$). One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 14$, $p < 0.01$) on the rate of serotonin metabolism measured by the index [5-HIAA]/[5-HT]x100 in the hippocampus (Table 2). Duncan's post hoc tests showed that the acute dose of ketamine significantly increased this index (~50%, $p < 0.01$), and this effect was completely blocked by both doses of 1MeTIQ ($p < 0.01$). In contrast, olanzapine administered alone and with ketamine elevated the rate of serotonin metabolism (by 40% and 60%, respectively, $p < 0.01$) (Table 2).

One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 4.06$, $p < 0.01$) on NA concentrations in the hippocampus (Table 2). Post hoc analysis demonstrated that an acute dose of ketamine significantly decreased the NA level in the hippocampus (~25%; $p < 0.05$). In addition, olanzapine given alone and with ketamine also reduced concentrations of NA (~30%; $p < 0.01$). A similar effect was observed when 1MeTIQ (50 mg/kg) was administered alone and with ketamine (~30%; $p < 0.01$) (Table 2). The statistical analysis demonstrated a significant effect of treatment ($F[7,38] = 3.61$, $p < 0.01$) on NM concentrations in the hippocampus (Table 2). Duncan's post hoc test showed that only in the 1MeTIQ (50 mg/kg) group was there a significant elevation in levels of NM (~30%; $p < 0.05$) (Table 2). One-way ANOVA showed a significant effect of treatment ($F[7,40] = 3.5$, $p < 0.01$) on the rate of noradrenaline metabolism measured by the index [NM] / [NA] × 100 in the hippocampus (Table 2). The post hoc analyses indicated that 1MeTIQ (50 mg/kg) administered alone and with ketamine, significantly increased this index (~100%, $p < 0.01$).

3.2.2.3. Striatum. One-way ANOVA indicated a significant effect of treatment ($F[7,40] = 8.52$, $p < 0.01$) on 5-HT concentrations in the striatum (Table 2). A post hoc test showed that the higher dose of 1MeTIQ (50 mg/kg) produced an elevation of the 5-HT concentration (~40%). A similar effect was observed when 1MeTIQ (25 mg/kg) was administered with ketamine (Table 2). In contrast, olanzapine with ketamine significantly decreased the level of 5-HT (~30%). The same statistical analysis showed a significant effect of treatment ($F[7,40] = 2.30$, $p < 0.05$) on levels of 5-HIAA (Table 2). One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 23.3$,

Table 2
The impact of 1MeTIQ and olanzapine (OLA) on ketamine-induced (KET) changes in serotonin (5-HT) and noradrenaline (NA) levels and their metabolism in the rat brain.

Frontal cortex

Treatment	5-HT (ng/g t)	5-HIAA (ng/g t)	NA (ng/g t)	NM (ng/g t)	5-HIAA/5-HT	NM/NA
Saline	421 ± 27	185 ± 7	351 ± 10	44 ± 7	45 ± 3	13 ± 2
KET_10	434 ± 19	210 ± 17	361 ± 9	44 ± 5	48 ± 2	12 ± 1
OLA_3	385 ± 23	202 ± 17	293 ± 11*++	37 ± 4	52 ± 4	13 ± 1
MeTIQ_25	570 ± 26**++	170 ± 9	362 ± 18	66 ± 7*+	30 ± 2**++	19 ± 3
MeTIQ_50	533 ± 17**++	147 ± 15*++	365 ± 17	98 ± 4**++	25 ± 2**++	27 ± 2**++
OLA3_KET	338 ± 23*++	215 ± 14	308 ± 13*+	27 ± 4	64 ± 4**++	9 ± 1
MeTIQ25_KET	551 ± 23**++	155 ± 14+	368 ± 19	61 ± 6	28 ± 2**++	17 ± 2
MeTIQ50_KET	487 ± 25	147 ± 15++	365 ± 17	85 ± 10**++	31 ± 4**++	24 ± 4**++
F	F(7/40) = 14,14 P < 0,01	F(7/40) = 5,19 P < 0,01	F(7/40) = 3,91 P < 0,01	F(7/40) = 15,7 P < 0,01	F(7/40) = 19,4 P < 0,01	F(7/40) = 7,9 P < 0,01

Hippocampus

Treatment	5-HT (ng/g t)	5-HIAA (ng/g t)	NA (ng/g t)	NM (ng/g t)	5-HIAA/5-HT	NM/NA
Saline	403 ± 107	339 ± 24	421 ± 43	70 ± 1	94 ± 10	18 ± 2
KET_10	257 ± 16*	350 ± 30	333 ± 35*	60 ± 15	136 ± 5**	21 ± 2
OLA_3	261 ± 24*	325 ± 27	253 ± 14**	41 ± 13	127 ± 9**	15 ± 4
MeTIQ_25	387 ± 9+	291 ± 8	371 ± 21	78 ± 8	82 ± 8++	22 ± 3
MeTIQ_50	289 ± 10	219 ± 19**++	308 ± 10**	113 ± 9*+	73 ± 4++	37 ± 4**+
OLA3_KET	264 ± 13*	382 ± 27	308 ± 21**	75 ± 4	146 ± 11**	26 ± 2
MeTIQ25_KET	314 ± 16	275 ± 25	328 ± 23*	92 ± 15	81 ± 5++	29 ± 5
MeTIQ50_KET	265 ± 15*	205 ± 27**++	292 ± 17**	96 ± 15	77 ± 8++	32 ± 6*
F	F(7/36) = 2,39 p < 0,05	F(7/40) = 6,71 p < 0,01	F(7/40) = 4,0 p < 0,01	F(7/38) = 3,61 p < 0,01	F(7/40) = 14 p < 0,01	F(7/40) = 3,5 p < 0,01

Striatum

Treatment	5-HT (ng/g t)	5-HIAA (ng/g t)	NA (ng/g t)	NM (ng/g t)	5-HIAA/5-HT	NM/NA
Saline	200 ± 8	288 ± 7	454 ± 18	110 ± 29	146 ± 9	23 ± 6
KET_10	196 ± 7	291 ± 20	443 ± 22	84 ± 19	148 ± 6	20 ± 5
OLA_3	192 ± 11	304 ± 17	434 ± 8	103 ± 12	158 ± 6	24 ± 3
MeTIQ_25	230 ± 14	243 ± 17	379 ± 14**	84 ± 10	106 ± 6**++	22 ± 3
MeTIQ_50	280 ± 16**	237 ± 14	439 ± 14	99 ± 6	85 ± 4**++	22 ± 1
OLA3_KET	150 ± 11*+	294 ± 18	399 ± 17*	84 ± 11	197 ± 4**++	21 ± 3
MeTIQ25_KET	255 ± 22*++	252 ± 24	444 ± 16	111 ± 13	100 ± 7**++	25 ± 3
MeTIQ50_KET	209 ± 15	243 ± 22	211 ± 15**++	58 ± 13*	119 ± 14*+	26 ± 5
F	$F_{(7/40)}$ = 8,52 p < 0,01	$F_{(7/40)}$ = 2,30 p < 0,05	$F_{(7/40)}$ = 25,39 p < 0,01	$F_{(7/40)}$ = 1,25 NS	$F_{(7/40)}$ = 23,3 p < 0,01	$F_{(7/40)}$ = 0,3 NS

Nucleus accumbens

Treatment	5-HT (ng/g t)	5-HIAA (ng/g t)	NA (ng/g t)	NM (ng/g t)	5-HIAA/5-HT	NM/NA
Saline	526 ± 17	409 ± 27	436 ± 88	2,5 ± 0,9	77 ± 4	0,7 ± 0,3
KET_10	509 ± 54	410 ± 31	1054 ± 97*	0,17 ± 0,03	82 ± 3	0,02 ± 0,001
OLA_3	345 ± 32*	362 ± 24	435 ± 68	0,50 ± 0,11	107 ± 7**++	0,1 ± 0,02
MeTIQ_25	635 ± 65	322 ± 24*	661 ± 162	0,25 ± 0,15	52 ± 4**++	0,04 ± 0,02
MeTIQ_50	687 ± 68*	322 ± 20*	1085 ± 109*	22,5 ± 2,22**	48 ± 2**++	2,2 ± 0,2**++
OLA3_KET	320 ± 26**+	397 ± 29	624 ± 173	0,10	125 ± 8**++	0,02 ± 0,01
MeTIQ25_KET	645 ± 66	357 ± 26	954 ± 254	0,43 ± 0,31	57 ± 5**++	0,05 ± 0,04
MeTIQ50_KET	625 ± 26	322 ± 21*+	1567 ± 284**+	27,3 ± 2,47**++	52 ± 3**++	2,2 ± 0,5**++
F	$F_{(7/40)}$ = 8,23 p < 0,01	$F_{(7/40)}$ = 2,31 p < 0,05	$F_{(7/40)}$ = 5,11 p < 0,01	$F_{(7/40)}$ = 85,48 p < 0,01	$F_{(7/40)}$ = 34,1 p < 0,01	$F_{(7/40)}$ = 17 p < 0,01

The rats received a single injection of saline (control), ketamine (KET) (10 mg/kg i.p.), 1MeTIQ in two doses (25 and 50 mg/kg i.p.) or olanzapine (OLA) (3 mg/kg i.p.). In the combination group, 1MeTIQ or OLA was given 20 min before the KET (10 mg/kg i.p.) injection. Data were analysed by one-way ANOVA followed by Duncan's post hoc tests. The data are means ± S.E.M. and the number of animals was n = 6; *p < 0.05 and **p < 0.01 indicate a difference from the control group (SAL); +p < 0.05 and ++p < 0.01 indicate a difference from the KET group.

p < 0.01) on the rate of serotonin metabolism measured by the index [5-HIAA]/[5-HT]x100 in the striatum (Table 2). Duncan's post hoc tests showed that both doses of 1MeTIQ given alone and with ketamine significantly reduced this index (up to 40%, p < 0.01). In contrast, olanzapine with ketamine elevated the rate of serotonin metabolism (by 30%, p < 0.01) (Table 2).

One-way ANOVA demonstrated a significant effect of treatment ($F[7,40] = 25.39$, $p < 0.01$) on NA concentrations in the striatum (Table 2). A significant decrease in concentrations of NA was observed after treatment with olanzapine (~15%), 1MeTIQ (25 mg/kg) (~20%) and 1MeTIQ (50 mg/kg) with ketamine (~50%). A statistical analysis revealed no significant effect of treatment ($F[7,40] = 1.25$, N.S.) on NM concentrations in the striatum (Table 2). One-way ANOVA showed no significant effect of treatment ($F[7,40] = 0.3$, N.S.) on the rate of noradrenaline metabolism measured by the index [NM] / [NA] × 100 in the striatum (Table 2).

3.2.2.4. Nucleus accumbens. One-way ANOVA showed a significant effect of treatment ($F[7,40] = 8.23$, $p < 0.01$) on 5-HT concentrations in the nucleus accumbens (Table 2). A post hoc test showed that an acute dose of 1MeTIQ (50 mg/kg) increased the concentration of 5-HT (~25%). while olanzapine given alone and with ketamine significantly reduced 5-HT levels (30% and 40%, respectively). The same statistical analysis demonstrated a significant effect of treatment ($F[7,40] = 2.31$, $p < 0.05$) on levels of 5-HIAA (Table 2). A significant decrease in concentrations of 5-HIAA was observed after treatment with both 1MeTIQ (25 and 50 mg/kg) and 1MeTIQ (50 mg/kg) with ketamine (~20%). One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 34.1$, $p < 0.01$) on the rate of serotonin metabolism measured by the index [5-HIAA]/[5-HT]x100 in the nucleus accumbens (Table 2). Duncan's post hoc test showed that 1MeTIQ (25 or 50 mg/kg) given alone and with ketamine significantly reduced this index (~20%, $p < 0.01$). At the same time, olanzapine administered alone and with ketamine elevated the rate of serotonin metabolism (by 30% and 40%, respectively, $p < 0.01$) in the nucleus accumbens (Table 2).

One-way ANOVA revealed a significant effect of treatment ($F[7,40] = 5.11$, $p < 0.01$) on NA concentrations in the nucleus accumbens (Table 2). Duncan's post hoc test showed that an acute dose of ketamine (10 mg/kg) produced a significant elevation in the NA concentration (~150%). A similar effect was observed after treatment with 1MeTIQ (50 mg/kg). In the 1MeTIQ (50 mg/kg) and ketamine group, this effect was stronger (~280%). The same statistical analysis demonstrated a significant effect of treatment ($F[7,40] = 85.48$, $p < 0.01$) on levels of NM (Table 2). Significant increases in NM concentrations were observed following 1MeTIQ (50 mg/kg) and 1MeTIQ (50 mg/kg) with ketamine (~1000%). One-way ANOVA showed a significant effect of treatment ($F[7,40] = 17$, $p < 0.01$) on the rate of noradrenaline metabolism measured by the index [NM] / [NA] × 100 in the nucleus accumbens (Table 2). The post hoc analysis indicated 1MeTIQ (50 mg/kg) alone and with ketamine, significantly increased this index (~300%, $p < 0.01$).

4. Discussion

Anxiety is a complex psychological phenomenon in which a number of brain structures and many different neurotransmitter systems are involved (Zarrindast and Khakpai, 2015). The main structures involved in anxiety-like behaviour are the hippocampus, prefrontal cortex, amygdala and nucleus accumbens. The main finding of the present study is that acute treatment with 1MeTIQ can block ketamine-induced anxiogenic effects on rat performance in the EPM test. This test is a simple screening test often used to measure anxiety-like behaviour in animal models (Becker et al., 2003; Pietersen et al., 2006; Horsley et al., 2018). Ketamine acts as a non-competitive antagonist of NMDA receptors, modulates dopamine and opioid transmission and has affinity for the serotonin transporter (SERT) (White and Ryan, 1996; Vollenweider, 2001). Ketamine at subanaesthetic doses may induce schizophrenia-like symptoms and promote anxiogenic behaviour (Babar et al., 2001; Frohlich and Van Horn, 2014). In our experiment, a low dose of ketamine (10 mg/kg i.p.) significantly reduced the percentage of time spent in the open arms of the apparatus (Fig. 1A). At the same time, the percentage of open arm entries was only weakly decreased (not significant) (Fig. 1B). These results clearly indicate the anxiogenic effects of ketamine. There is evidence that the behavioural effects of ketamine depend on the dose used and the method of ketamine administration (Becker et al., 2003; da Silva et al., 2010; Horsley et al., 2018). In the EPM test, acute treatment with a higher dose of 1MeTIQ (50 mg/kg) completely blocked the ketamine-induced anxiogenic effect. In the 1MeTIQ-treated group, the percentage of time spent in the open arms was similar to that in the control group (Fig. 1A). A similar anxiolytic-like effect was observed when olanzapine was administered with ketamine (Fig. 1A).

The Any-maze® tracking system used in the present study also measured the locomotor activity of the rats (the total number of entries and the number of rearings). In our experiment, a low dose of ketamine did not change any of the measured parameters (Fig. 1C and D). However, 1MeTIQ (50 mg/kg) or olanzapine (3 mg/kg) administered with ketamine significantly decreased both measures of locomotor activity, the total number of entries (Fig. 1C) and the number of rearings (Fig. 1D). Moreover, olanzapine given alone also reduced both parameters. Such an effect of olanzapine on the locomotor activity of rats has been described by other authors (Rogóż and Skuza, 2011). The increase in the percentage of time spent in the open arms, despite the decreases in the motor activity of the animals, testifies to the anxiolytic-like effect of 1MeTIQ. As we have previously demonstrated, 1MeTIQ does not exhibit psychostimulant properties despite increasing dopamine release (Wąsik et al., 2013; Wąsik et al., 2016). In the present ex vivo biochemical analyses, a low dose of ketamine did not change the levels of dopamine or its metabolites (Table 1). However, other authors have indicated that acute ketamine administration (at a higher dose) produces synaptic depression in different structures (the prefrontal cortex, hippocampus and nucleus accumbens) via dopamine D_1/D_5 receptors through increased dopamine release (Duan et al., 2013; Hunt et al., 2005; Kamiyama et al., 2011; Lorrain et al., 2003). Several studies have reported that the excessive activation of D_1/D_5 receptors worsens memory (Vijayraghavan et al., 2007; Wang et al., 2012; Zahrt et al., 1997). The modulating effect of 1MeTIQ on various neurotransmitter systems in the mammalian brain is well documented (Antkiewicz-Michaluk et al., 2006; Antkiewicz-Michaluk et al., 2014; Kuszczyk et al., 2010; Wąsik et al., 2013; Wąsik et al., 2014). 1MeTIQ acts as a dopamine receptor partial agonist and may play an important physiological role as an inhibitory regulator that counteracts the excessive stimulation of the catecholaminergic system (Antkiewicz-Michaluk et al., 2007). As a reversible inhibitor of MAO activity (Patsenka and Antkiewicz-Michaluk, 2004), 1MeTIQ strongly influences the catabolism of dopamine, serotonin and norepinephrine. Some clinical studies have indicated that anxiety disorders are mediated by both the serotoninergic and dopaminergic systems (see review Williams et al., 2017). The efficacy of the anxiolytic effects of MAO inhibitors in clinical trials was demonstrated in the 1990s (Fahlen et al., 1995; Van Vliet et al., 1992; Versiani et al., 1992). It is important that reversible MAO inhibitors are better tolerated and safer than irreversible MAO inhibitors (Versiani et al., 1992). 1MeTIQ, as a reversible MAO inhibitor, increases the concentration of biogenic amines by blocking their catabolism by the MAO-dependent oxidation pathway and instead allowing them to be methylated in a COMT-dependent manner. One effect of 1MeTIQ is the three times increase in the concentration of 3-MT, an extraneuronal metabolite of dopamine that is present in the hippocampus, striatum and nucleus accumbens (but not in the frontal cortex) (Table 1). 3-MT is a biologically active compound that may play a role as a possible regulator of the activity of the catecholaminergic system and is an antagonist of the behavioural and neurochemical effects induced by the systemic administration of amphetamine (Antkiewicz-Michaluk et al., 2008). In vitro binding studies have also demonstrated that 3-MT possesses a considerable affinity for noradrenergic and dopaminergic receptors (particularly for the noradrenergic alpha1 receptor) (Antkiewicz-Michaluk et al., 2008).

Considering the role of noradrenergic receptors in anxiety (Morilak et al., 2005; Ehlers and Todd, 2017), we postulate that 1MeTIQ administered at a higher dose of 50 mg/kg, as is used in the present paper, may possess anxiolytic-like effects by increasing the levels of 3-MT, an antagonist of the noradrenergic alpha1 receptor. Moreover, 1MeTIQ, by inhibiting the oxidation pathway, exerts a beneficial effect by reducing oxidative stress in dopaminergic neurons. Such an anti-oxidative effect of 1MeTIQ can be seen clearly in the striatum and nucleus accumbens (Table 1). In both structures, the concentration of DOPAC, as a product of MAO-dependent oxidation, was essentially reduced, while the level of 3-MT, the final product of COMT-dependent methylation, was elevated many times by a higher dose of 1MeTIQ.

At the same time, it should be emphasized that olanzapine administered alone as well as with ketamine has a strong pro-oxidative effect, which is undesirable and leads to a strong increase in the DOPAC concentration (above 300%), which is always connected with the excessive production of free radicals, in the investigated brain structures. In addition, the rate of total dopamine metabolism, as measured by the index [HVA]/[DA], was also significantly increased (Table 1). A similar effect of olanzapine on the essential activation of dopamine release was observed in an in vivo microdialysis study (Ratajczak et al., 2016a, 2016b). In clinical studies, some authors have demonstrated an unproductive influence of olanzapine on the function of the liver (Bilgic et al., 2017).

The biochemical data in Table 2 demonstrate that a low dose of ketamine significantly decreased the 5-HT concentration in the rat hippocampus. 1MeTIQ, similar to olanzapine, did not change this effect of ketamine. However, 1MeTIQ (50 mg/kg) administered alone or with ketamine significantly decreased the levels of 5-HIAA. This means that the catabolism of serotonin, as measured by the index [5-HIAA] / [5-HT], was inhibited due to the blockade of MAO activity and that there was a consequent increased serotonin concentration in synapses. In contrast, olanzapine administered alone or with ketamine produced an elevation of the total rate of serotonin catabolism. As described by Graeff et al. (1996), serotonin plays an important role in modulating the phenomenon of anxiety. Serotonin has anxiolytic properties; however, the regulation of the serotonergic system is very complicated (Deakin and Graeff, 1991). Undoubtedly, increasing the release of serotonin has beneficial effects in the context the anxiolytic-like properties of 1MeTIQ.

Olanzapine is an atypical neuroleptic and acts as an antagonist of dopamine (D_2, D_3, D_4), serotonin ($5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$, $5\text{-}HT_6$) and noradrenergic alpha1 receptors (Angelucci et al., 2005). Therefore, the mechanism of actions of 1MeTIQ and olanzapine are rather different. While 1MeTIQ affects the catabolism of monoamines, olanzapine acts mainly by inhibiting monoamine receptors; however, the antagonism of noradrenergic alpha1 receptors is a common feature of both of the investigated substances and may contribute to their anxiolytic-like properties. An in vivo microdialysis study indicated that olanzapine elevates the release of both dopamine and serotonin in the frontal cortex in schizophrenic-like rats (Ratajczak et al., 2016a, 2016b). In our ex vivo neurochemical study, acute ketamine administration significantly influenced noradrenaline levels but caused opposite effects in different rat brain structures; in the hippocampus, we observed a decrease in noradrenaline levels, whereas in the nucleus accumbens, we observed a significant increase in the level of this neurotransmitter (Table 2). 1MeTIQ administered with ketamine did not change these described effects. There is strong evidence that noradrenaline systems are involved in anxiety (McCall et al., 2015; Purvis et al., 2018; Tanaka et al., 2000). For example, increasing the firing of noradrenergic neurons is itself anxiogenic (McCall et al., 2015). Moreover, stress causes a significant increase in noradrenaline release in different brain structures (Glavin et al., 1983; Tanaka et al., 1982; Tanaka et al., 1983; Tanaka et al., 1985). It has been clearly demonstrated that drugs increasing noradrenaline release, such as yohimbine, enhance anxiety-like behaviour (Charney et al., 1983). In the present paper, we measured the concentration of NM, a direct extraneuronal metabolite of noradrenaline, in different brain structures. In the nucleus accumbens, ketamine produced a significant decrease in the level of NM, while 1MeTIQ at a higher dose (50 mg/kg) alone or together with ketamine caused a massive increase in the level of this metabolite (Table 2). This effect suggests that ketamine may inhibit COMT activity and consequently lead to an increase in the concentration of noradrenaline in synapses (Lundy and Frew, 1981; Robinson et al., 2016). 1MeTIQ leads to a considerable increase in the rate of the catabolism of noradrenaline, as measured by the index [NM]/[NA]. As a result, the amount of noradrenaline in the synaptic cleft may be reduced compared to that in the ketamine alone group. Such opposing mechanisms of action of ketamine and 1MeTIQ on COMT activity can also contribute to the anxiogenic-like effect of ketamine and the anxiolytic-like effect of 1MeTIQ.

5. Conclusion

The above data clearly indicate that acute treatment with 1MeTIQ at a high dose has anxiolytic-like effects in the EPM test similar to those of olanzapine. The mechanism responsible for the anxiolytic-like effect of 1MeTIQ is related to its influence on dopamine catabolism (especially the increase of 3-MT, an extraneuronal dopamine metabolite that possesses antagonistic properties to the noradrenergic alpha1 receptor), as well as increase in serotonin concentrations and decreases in the levels of noradrenaline. We believe that further microdialysis studies will be able to explain the influence of acutely administered ketamine and 1MeTIQ on the in vivo release of monoamines and glutamate in the rat brain.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Angelucci, F., Aloe, L., Iannitelli, A., Gruber, S.H., Mathe, A.A., 2005. Effect of chronic olanzapine treatment on nerve growth factor and brain-derived neurotrophic factor in the rat brain. Eur. Neuropsychopharmacol. 15 (3), 311–317.

Antkiewicz-Michaluk, L., Lazarewicz, J.W., Patsenka, A., Kajta, M., Zieminska, E., Salinska, E., Wasik, A., Golembiowska, K., Vetulani, J., 2006. The mechanism of 1,2,3,4-tetrahydroisoquinolines neuroprotection: the importance of free radicals scavenging properties and inhibition of glutamate-induced excitotoxicity. J. Neurochem. 97 (3), 846–856.

Antkiewicz-Michaluk, L., Filip, M., Michaluk, J., Romańska, I., Przegaliński, E., Vetulani, J., 2007. An endogenous neuroprotectant substancje, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ), prevents the behavioral and neurochemical effects of cocaine reinstatement in drug-dependent rats. J. Neural Transm. 114, 307–317.

Antkiewicz-Michaluk, L., Ossowska, K., Romańska, I., Michaluk, J., Vetulani, J., 2008. 3-Methoxytyramine, an extraneuronal dopamine metabolite plays a physiological role in the brain as an inhibitory regulator of catecholaminergic activity. Eur. J. Pharmacol. 599, 32–35.

Antkiewicz-Michaluk, L., Wąsik, A., Michaluk, J., 2014. 1-Methyl-1,2,3,4-tetrahydroisoquinoline, an endogenous amine with unexpected mechanism of action: new vistas of therapeutic application. Neurotox. Res. 25 (1), 1–12.

Arakawa, R., Ito, H., Okumura, M., Takano, A., Takahashi, H., Takano, H., Okubo, Y., Suhara, T., 2010. Extrastriatal dopamine D2 receptor occupancy in olanzapine-treated patients with schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci. 260 (4), 345–350.

Babar, E., Ozgunen, T., Melik, E., Polat, S., Akman, H., 2001. Effects of ketamine on different types of anxiety. Eur. J. Pharmacol. 431 (3), 315–320.

Becker, A., Peters, B., Schroeder, H., Mann, T., Huether, G., Grecksch, G., 2003. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 27 (4), 687–700.

Bilgic, S., Tastemir Korkmaz, D., Azirak, S., Guvenc, A.N., Kocaman, N., Ozer, M.K., 2017. The protective effect of thymoquinone over olanzapine-induced side effects in liver, and metabolic side effects. Bratisl. Med. J. 118 (10), 618–625.

Braga, R.J., Reynolds, G.P., Siris, S.G., 2013. Anxiety comorbidity in schizophrenia. Psychiatry Res. 210 (1), 1–7.

Charney, D.S., Heninger, G.R., Redmond Jr., D.E., 1983. Yohimbine induced anxiety and increased noradrenergic function in humans: effects of diazepam and clonidine. Life Sci. 33 (1), 19–29.

Costa, A.A., Morato, S., Roque, A.C., Tinós, R., 2014. A computational model for exploratory activity of rats with different anxiety levels in elevated plus-maze. J. Neurosci. Methods 236, 44–50.

. da Silva F. C., do Carmo de Oliveira Cito, M., da Silva M. I., Moura B. A., de Aquino Neto M. R., Feitosa M. L., de Castro Chaves R., Macedo D. S., de Vasconcelos S. M., de França Fonteles M. M., de Sousa F. C., 2010. Behavioral alterations and pro-oxidant effect of a single ketamine administration to mice. Brain Res. Bull. 83 (1–2), 9–15.

Deakin, J.F., Graeff, F.G., 1991. 5-HT and mechanisms of defence. J. Psychopharmacol. 5 (4), 305–315.

Duan, T.T., Tan, J.W., Yuan, Q., Cao, J., Zhou, Q.X., Xu, L., 2013. Acute ketamine induces hippocampal synaptic depression and spatial memory impairment through dopamine D1/D5 receptors. Psychopharmacology 228 (3), 451–461.

Ehlers, M.R., Todd, R.M., 2017. Genesis and maintenance of attentional biases: the role of the locus coeruleus-noradrenaline system. Neural Plast. 2017, 6817349.

Fahlen, T., Nilsson, H.I., Borg, K., Humble, M., Pauli, U., 1995. Social phobia: the clinical efficacy and tolerability of the monoamine oxidase-A and serotonin uptake inhibitor brofaromine. A double-blind placebo-controlled study. Acta Psychiatr. Scand. 92 (5), 351–358.

Frohlich, J., Van Horn, J.D., 2014. Reviewing the ketamine model for schizophrenia. J. Psychopharmacol. 28 (4), 287–302.

Glavin, G.B., Tanaka, M., Tsuda, A., Kohno, Y., Hoaki, Y., Nagasaki, N., 1983. Effects of altered brain noradrenaline level on acute stress pathology in rats. Kurume Med. J. 30, 31–34.

Graeff, F.G., Guimarães, F.S., De Andrade, T.G., Deakin, J.F., 1996. Role of 5-HT in stress, anxiety, and depression. Pharmacol. Biochem. Behav. 54 (1), 129–141.

Griebel, G., Holmes, A., 2013. 50 years of hurdles and hope in anxiolytic drug discovery. Net. Rev. Drug Discov. 12 (9), 667–687.

Horsley, R.R., Páleníček, T., Kolin, J., Valeš, K., 2018. Psilocin and ketamine microdosing: effects of subchronic intermittent microdoses in the elevated plus-maze in male Wistar rats. Behav. Pharmacol. 29 (6), 530–536.

Hunt, M.J., Kessal, K., Garcia, R., 2005. Ketamine induces dopamine-dependent depression of evoked hippocampal activity in the nucleus accumbens in freely moving rats. J. Neurosci. 25 (2), 524–531.

Kamiyama, H., Matsumoto, M., Otani, S., Kimura, S.I., Shimamura, K.I., Ishikawa, S., Yanagawa, Y., Togashi, H., 2011. Mechanisms underlying ketamine-induced synaptic depression in rat hippocampus-medial prefrontal cortex pathway. Neuroscience 177, 159–169.

Koehntop, D.E., Liao, J.C., Van Bergen, F.H., 1977. Effects of pharmacologic alterations of adrenergic mechanisms by cocaine, tropolone, aminophylline, and ketamine on epinephrine-induced arrhythmias during halothane-nitrous oxide anesthesia. Anesthesiology 46 (2), 83–93.

Komossa, K., Rummel-Kluge, C., Hunger, H., Schmid, F., Schwarz, S., Duggan, L., Kissling, W., Leucht, S., 2010. Olanzapine versus other atypical antipsychotics for schizophrenia. Cochrane Database Syst. Rev. 17.

Krystal, J.H., D'Souza, D.C., Karper, L.P., Bennet, A., Abi-Dargham, A., Abi Saab, D., Cassello, K., Bowers Jr., M.B., Vegso, S., Heninger, G.R., Charney, D.S., 1999. Interactive effects of subanesthetic ketamine and haloperidol an healthy humans. Psychopharmacology 145, 193–204.

Kulhan, T., Marcincakova Husarova, V., Ondrejka, I., Farsky, I., Nosalova, G., 2018. Anxiolytic effects of venlafaxine/olanzapine combination in treatment of anxious depression. Neuro Endocrinol. Lett. 38 (8), 579–585.

Kuszczyk, M., Słomka, M., Antkiewicz-Michaluk, L., Salińska, E., Łazarewicz, J.W., 2010. 1-Methyl-1,2,3,4-tetrahydroisoquinoline and established uncompetitive NMDA receptor antagonists induce tolerance to excitotoxicity. Pharmacol. Rep. 62 (6), 1041–1050.

Kuszczyk, M.A., Sadowski, M.J., Antkiewicz-Michaluk, L., Lazarewicz, J.W., 2014. 1MeTIQ provides protection against Aβ-induced reduction of surface expression of synaptic proteins and inhibits H_2O_2-induced oxidative stress in primary hippocampal neurons. Neurotox. Res. 25 (4), 348–357.

Lian, J., Huang, X.F., Pai, N., Deng, C., 2013. Effects of olanzapine and betahistine co-treatment on serotonin transporter, 5-HT2A and dopamine D2 receptor binding density. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 47, 62–68.

Lorrain, D.S., Baccei, C.S., Bristow, L.J., Anderson, J.J., Varney, M.A., 2003. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 117 (3), 697–706.

Lundy, P.M., Frew, R., 1981. Ketamine potentiates catecholamine responses of vascular smooth muscle by inhibition of extraneuronal uptake. Can. J. Physiol. Pharmacol. 59 (6), 520–527.

McCall, J.G., Al-Hasani, R., Siuda, E.R., Hong, D.Y., Norris, A.J., Ford, C.P., Bruchas, M.R., 2015. CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. Neuron 87 (3), 605–620.

Mendonca Jùnior, F.J., Scotti, L., Ishiki, H., Botelho, S.P., Da Silva, M.S., Scotti, M.T., 2015. Benzo- and thienobenzo-diazepines: multi target drugs for CNS disorders. Mini Rev. Med. Chem. 15 (8), 630–647.

Morilak, D.A., Barrera, G., Echevarria, D.J., Garcia, A.S., Hernandez, A., Ma, S., Petre, C.O., 2005. Role of brain norepinephrine in the behavioral response to stress. Prog. Neuro-Psychopharmacol. 29, 1214–1224.

Nagai, N., Watanabe, K., 2013. Olanzapine. Nihon Rinsho 71 (4), 666–672.

Nikiforuk, A., Kos, T., Wesołowska, A., 2011. The 5-HT6 receptor agonist EMD 386088 produces antidepressant and anxiolytic effects in rats after intrahippocampal administration. Psychopharmacology 217 (3), 411–418.

Pan, P.Y., Lee, M.S., Lo, M.C., Yang, E.L., Yeh, C.B., 2014. Olanzapine is superior to lamotrigine in the prevention of bipolar depression: a naturalistic observational study. BMC Psychiatry 14, 145.

Patsenka, A., Antkiewicz-Michaluk, L., 2004. Inhibition of the rodent brain monoamine oxidase and tyrosine hydroxylase by endogenous compounds-1,2,3,4-tetrahydroisoquinoline alkaloids. Pol. J. Pharmacol. 56, 727–734.

Pietersen, C.Y., Bosker, F.J., Postema, F., Fokkema, D.S., Korf, J., den Boer, J.A., 2006. Ketamine administration disturbs behavioural and distributed neural correlates of fear conditioning in the rat. Prog. Neuropsychopharmacol. Biol. Psychiatry 30 (7), 1209–1218.

Purvis, E.M., Klein, A.K., Ettenberg, A., 2018. Lateral habenular norepinephrine contributes to states of arousal and anxiety in male rats. Behav. Brain Res. 347, 108–115.

Ratajczak, P., Kus, K., Giermaziak, W., Nowakowska, E., 2016a. The influence of aripiprazole and olanzapine on the anxiolytic-like effect observed in prenatally stressed rats (animal model of schizophrenia) expose to the ethyl alcohol. Pharmacol. Rep. 68, 415–422.

Ratajczak, P., Kus, K., Gołembiowska, K., Noworyta-Sokołowska, K., Woźniak, A., Zaprutko, T., Nowakowska, E., 2016b. The influence of aripiprazole and olanzapine on neurotransmitters level in front al cortex of prenatally stressed rats. Environ. Toxicol. Pharmacol. 46, 122–130.

Robinson, B.L., Dumas, M., Cuevas, E., Gu, Q., Paule, M.G., Ali, S.F., Kanungo, J., 2016. Distinct effects of ketamine and acetyl L-carnitine on the dopamine system in zebrafish. Neurotoxicol. Teratol. 54, 52–60.

Rogóż, Z., Skuza, G., 2011. Anxiolytic-like effects of olanzapine, risperidone and fluoxetine in the elevated plus-maze test in rats. Pharmacol. Rep. 63 (6), 1547–1552.

Silvestre, J.S., Nadal, R., Pallarés, M., Ferrés, N., 1997. Acute effects of ketamine in the holeboard, the elevated-plus maze, and the social interaction test in Wistar rats. Depress. Anxiety 5 (1), 29–33.

Suppes, T., Eberhard, J., Lemming, O., Young, A.H., McIntyre, R.S., 2017. Anxiety, irritability and agitation as indicators of bipolar mania with depressive symptoms: a post hoc analysis of two clinical trials. Int. J. Bipolar Disord. 5 (36).

Tanaka, M., Kohno, Y., Nakagawa, R., Ida, Y., Iimori, K., Hoaki, Y., Tsuda, A., Nagasaki, N., 1982. Naloxone enhances stress-induced increases in noradrenaline turnover in specific brain regions in rats. Life Sci. 30 (19), 1663–1669.

Tanaka, M., Kohno, Y., Nakagawa, R., Ida, Y., Takeda, S., Nagasaki, N., Noda, Y., 1983. Regional characteristics of stress-induced increases in brain noradrenaline release in rats. Pharmacol. Biochem. Behav. 19 (3), 543–547.

Tanaka, M., Tsuda, A., Ida, Y., Ushijima, I., Tsujimaru, S., Nagasaki, N., 1985. Methionine-enkephalin inhibits stress-induced increases in noradrenaline turnover in brain regions of rats. Jpn. J. Pharmacol. 37 (117), 117–119.

Tanaka, M.M., Yoshida, M., Emoto, H., Ishii, H., 2000. Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies. Eur. J. Pharmacol. 405 (1–3), 397–406.

Van Vliet, I.M., Boer, J.A., Westenberg, H.G., 1992. Psychopharmacological treatment of social phobia: clinical and biochemical effects of brofaromine, a selective MAO-A inhibitor. Eur. Neuropsychopharmacol. 2 (1), 21–29.

Versiani, M., Nardi, A.E., Mundim, F.D., Alves, A.B., Liebowitz, M.R., Amrein, R., 1992. Pharmacotherapy of social phobia. A controlled study with moclobemide and phenelzine. Brit. J. Psychiat. 161, 353–360.

Vijayraghavan, S., Wang, M., Birnbaum, S.G., Williams, G.V., Arnsten, A.F., 2007. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat. Neurosci. 10, 376–384.

Vollenweider, F.X., 2001. Brain mechanisms of hallucinogens and entactogens. Dialogues Clin. Neurosci. 3 (4), 265–279.

Wang, Y., Wu, J., Zhu, B., Li, C., Cai, J.X., 2012. Dopamine D1 receptors are responsible for stress-induced emotional memory deficit in mice. Stress 15 (2), 237–242.

Wąsik, A., Romańska, I., Antkiewicz-Michaluk, L., 2010. Important role of 3-methoxytyramine in the inhibition of cocaine sensitization by 1-methyl-1,2,3,4-tetrahydroisoquinoline: an in vivo microdialysis study. Pharmacol. Rep. 62 (6), 983–997.

Wąsik, A., Możdżeń, E., Romańska, i., Michaluk, J., Antkiewicz-Michaluk, L., 2013. Antidepressant-like activity of the endogenous amine, 1-methyl-1,2,3,4-tetrahydroisoquinoline in the behavioral despair test in the rat, and its neurochemical correlates: a comparison with the classical antidepressant, imipramine. Eur. J. Pharmacol. 700 (1–3), 110–117.

Wąsik, A., Możdżeń, E., Michaluk, J., Romańska, I., Antkiewicz-Michaluk, L., 2014. 1-Methyl-1,2,3,4-tetrahydroisoquinoline, an endogenous neuroprotectant and MAO inhibitor with antidepressant-like properties in the rat. Neurotox. Res. 25 (4), 323–334.

Wąsik, A., Romańska, I., Antkiewicz-Michaluk, L., 2016. Comparison of the effects of acute and chronic administration of tetrahydroisoquinoline amines on the in vivo dopamine release: a microdialysis study in the rat striatum. Neurotox. Res. 30 (4), 648–657.

Wąsik, A., Romańska, I., Zelek-Molik, A., Antkiewicz-Michaluk, L., 2018. Multiple

administration of endogenous amines TIQ and 1MeTIQ protects against a 6-OHDA-induced essential fall of dopamine release in the rat striatum: in vivo microdialysis study. Neurotox. Res. 33 (3), 523–531.
White, J.M., Ryan, C.F., 1996. Pharmacological properties of ketamine. Drug Alcohol Rev. 15 (2), 145–155.
Williams, T., Hattingh, C.J., Kariuki, C.M., Tromp, S.A., van Balkom, A.J., Ipser, J.C., Stein, D.J., 2017. Pharmacotherapy for social anxiety disorder (SAnD). Cochrane Database Syst. Rev. 10.
Zahrt, J., Taylor, J.R., Mathew, R.G., Arnsten, A.F., 1997. Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. J. Neurosci. 17 (21), 8528–8535.
Zarrindast, M.R., Khakpai, F., 2015. The modulatory role of dopamine in anxiety-like behavior. Arch. Iran. Med. 18 (9), 591–603.