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Original article

Antidepressant-like effect of 1,2,3,4-tetrahydroisoquinoline and its methyl derivative in animal models of depression

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ABSTRACT

Background: Most of the currently used antidepressant drugs are monoamine-based compounds, acting by the inhibition of re-uptake or metabolism of noradrenaline (NA) and/or serotonin (5-HT), because these neurotransmitters play a key role in the pathophysiology of depression. The aim of this study was to investigate the potential antidepressant-like activity of an endogenous amine, 1,2,3,4-tetrahydroisoquinoline (TIQ) and its close derivative, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ).
Methods: The experiments were carried out on male C57BL6J mice. The antidepressant-like activity of TIQs was evaluated in the behavioral tests: the forced swim test (FST) and tail suspension test (TST) and neurochemical analysis. TIQ and 1MeTIQ were administrated in three differences doses of 10, 25 or 50 mg/kg. Imipramine (IMI; 15 or 30 mg/kg) was used as a reference drug. In the neurochemical *ex vivo* study, the levels of NA, 5-HT and their metabolites, the rate of monoamine metabolism and their neuronal activity in different mouse brain structures were determined by HPLC with electrochemical detection.
Results: The results of this study have demonstrated that TIQ and 1MeTIQ produced antidepressant-like effect in the FST and TST because they significantly decreased the immobility time comparably to IMI. Biochemical data have demonstrated that administration of TIQs led to the activation of NA and 5-HT systems.
Conclusions: The results reported in this paper indicate that TIQ and 1MeTIQ possess a distinct antidepressant activity. In the light of these findings, we suggest that both tested compounds may be effective for the depression therapy in a clinical setting with better tolerance of side effects.

Introduction

Depression is one of the most common diseases worldwide associated with a high rate of suicides. Despite intensive research the etiology and pathogenesis of depression remains unclear. Preclinical and clinical studies suggest that monoamine neurotransmitters such as dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in the central nervous system play a key role in the pathophysiology of depression [1–3]. These studies have focused largely on the levels of monoamines and their receptors, and have led to several theories of depression, including the monoamine depletion and receptor sensitivity hypothesis theories [4–6]. Despite the advances in the treatment of depression with selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) [7,8], there continue to be many unmet clinical needs with respect to both efficacy and side effects [9]. Recent advances in molecular and cellular neurobiology have provided new insights into the long-term adaptations that underlie the therapeutic action of antidepressant medications [10–13]. Effective drugs for depression are MAO inhibitors and/or 5-HT and NA reuptake inhibitors [7–9]. Although there has been a lot of efforts in the development of a new drugs in the last years the situation still is unsatisfying. The results and strategies discussed provide a framework for future studies, at the basic to further characterize the pathophysiology and treatment in depression. Finally, a more complete understanding of depression will be dependent on critical future studies, the suitable animal models to identify the additional intracellular pathways involved in the mechanism of this complex psychiatric disorder, and can greatly push our knowledge forward. To address these needs, antidepressants with novel mechanisms of action and without side effects are in great demand.

1,2,3,4-Tetrahyroisoquinoline (TIQ) and its methyl derivative, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) are members of

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tetrahydroisoquinoline family (TIQs). They are the most numerous alkaloids widespread in plants, a variety of food products as well as in the human, primate and rodent brain [14–18]. In most cases, TIQs can be formed as condensation products of biogenic amines (i.e., phenylethylamines and catecholamines) with aldehydes or α-keto acids by the so-called Pictet-Spengler reaction [14,17,19], although some of them for example 1MeTIQ may be also synthesized enzymatically in the brain [20–23]. TIQ and 1MeTIQ easily penetrate into the brain through the blood brain barrier with the high affinity for the brain tissue [24,25]. Exogenous TIQs was actively transported from the blood into the brain by organic cation transporter system. What is interesting, the concentration of TIQs in the brain was several-fold higher than in plasma both after acute and chronic administration. A half-life of TIQ in the rat brain was $t_{1/2}$ = 3 h 58 min while the respective value in the plasma was $t_{1/2}$ = 2 h 38 min [24]. What is important to mention is that both of these substances express a significant neuroprotective activity as demonstrated by recent extensive *in vitro* and *in vivo* experiments [26–29]. They have structural similarities to DA and can interact with agonistic conformation of DA receptors as partial agonists what makes their behavioral profile different from the typical neuroleptics [29–31]. Additionally, it has been found that TIQ and 1MeTIQ in low micro molar concentrations inhibit enzymatic activity of both isoforms of monoamine oxidase A (MAO A) and B (MAO B). Consequently, they inhibit the main catabolic pathway of DA, MAO-dependent oxidative deamination of DA, the route by which free oxygen radicals are generated and increase monoamine neurotransmitter levels in the brain [30,32–34]. Simultaneously, they shift DA catabolism towards COMT-dependent *O*-methylation what has essential neuroprotective significance [30,35]. What is more, as demonstrated in preclinical investigations in the 1970s, MAO inhibitors showed antidepressant-like properties [36]. Several short-acting and reversible MAO A and MAO B inhibitors are now under evaluation or in use as antidepressants, e.g. brofaromine, moclobemide [37].

The aim of the present study was to investigate the antidepressant-like effect of TIQ and its neuroprotective methyl derivative, 1MeTIQ in comparison with the classical tricyclic antidepressant, imipramine. The forced swim test (FST) and tail suspension test (TST) were used to examine their antidepressant-like activity in mice. The FST and TST are the screening tests with sensitivity to a short-term drug administration and high predictivity of antidepressant efficacy in human depression. Additionally, the locomotor activity test was used to check the motor function of mice after administration of the investigated compounds.

In addition to behavioral experiments, we also carried out a neurochemical *ex vivo* study in different mouse brain structures to determine the level of monoamines and their metabolites as well as the rate of monoamine (NA and 5-HT) metabolism and indicators of neuronal activity.

Materials and methods

Animals

The behavioral experiments were carried out on male C57BL6J mice (25 ± 2 g) (Charles River Laboratories, Sulzfeld, Germany). The animals were housed 5–8 per cage (57 × 35 × 20 cm) in a colony room maintained at 21 ± 1 °C and with a 40–50% humidity under an artificial day-night cycle (12/12 h, the light on at 7 a.m.). The animals had free access to standard laboratory food and tap water before the experiment. All the procedures were conducted during the light phase.

All the experimental protocols were approved by the Local Bioethics Commission for Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences in Krakow. All efforts were made to minimize animal suffering and the number of animals used.

Chemicals

TIQ (1,2,3,4-tetrahydroisoquinoline hydrochloride, Sigma-Aldrich, USA) and IMI (imipramine hydrochloride, Sigma-Aldrich, USA) were obtained commercially. 1MeTIQ (1-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride) was synthesized at the Medicinal Chemistry Department Institute of Pharmacology Polish Academy of Sciences. Purity of the compound was verified by measurement of the melting point, and homogeneity was assessed on a chromatographic column. All the investigated compounds were dissolved in a sterile 0.9% NaCl solution and injected intraperitoneally (*ip*). The chemical structures of the TIQ, 1MeTIQ and IMI are shown in Fig. 1.

Behavioral experiments

Behavior despair study

For the forced swim test (FST) animals were divided into nine groups (n = 6-7 animals/per group): Control (0.9% saline), IMI 30 mg/kg, and three doses of TIQ (10, 25, 50 mg/kg) or 1MeTIQ (10, 25, 50 mg/kg).

For the tail suspension test (TST), animals were divided into six groups (n = 8 animals/per group): Control (0.9% saline), IMI 15 mg/kg, and two doses of TIQ (10, 25 mg/kg) or 1 MeTIQ (10, 25 mg/kg).

All investigated compounds were administrated once 60 min before a behavioral test.

Forced swim test (FST)

The procedure was carried out on mice according to the method of Porsolt [38]. Briefly, each mouse was placed individually in an open cylindrical container (diameter: 10 cm, height: 25 cm) filled with water up to 9 cm at 22 ± 1 °C. The immobility time was

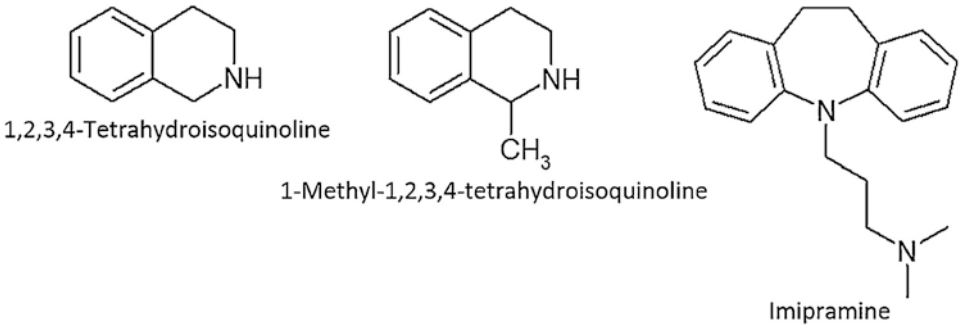

Fig. 1. The chemical structure of TIQ, 1MeTIQ and IMI.

recorded during the last 4 min of the 6-min testing period [39]. The immobility time was defined as the absence of active/escape directed movements (mouse floating in the water without struggling) and was scored in a blind manner by an observer [40]. A decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity. The animals were used only once in each experiment.

Tail suspension test (TST)

The total duration of immobility in the course of tail suspension was measured according to the method of Steru [39]. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The time during which mice remained immobile was quantified during a test period of 6 min. Mice were considered immobile only when hung passively and completely motionless.

Locomotor activity test

For the locomotor activity test, animals were divided into six groups (n = 7–8/group): Control (0.9% saline), IMI 30 mg/kg, and two different doses of TIQ (10, 25 mg/kg) or 1MeTIQ (10, 25 mg/kg) administered 60 min before the locomotor activity test.

The locomotor activity of mice was recorded using the Opto-M3 System (Columbus Instruments, Columbus, OH, USA), which is a multi-channel activity monitor that supports sensors (0.5" beam spacing) attached to the computer, which measures both ambulatory activity and total counts every min for 6 min. Each group consisted of 8 mice.

Biochemical ex vivo *studies*

The animals were killed by the cervical dislocation after the end of behavioral experiments (FST). The brains were rapidly removed and dissected on an ice-cold glass plate. After decapitation the substantia nigra, striatum, frontal cortex and hypothalamus were taken and immediately frozen on solid CO_2 (−70 °C) till used for biochemical assay. Noradrenaline (NA) and its metabolites, normetanephrine (NMN) and 3-methoxy-4-hydroxyphenylglycol (MHPG); serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were assayed by means of high-performance liquid chromatography (HPLC) with electrochemical detection (ED). The chromatograph (HP 1050; Hewlett-Packard, Golden, CO, USA) was equipped with C18 column.

The tissue samples were weighed and homogenized in ice-cold 0.1 M perchloroacetic acid containing 0.05 mM ascorbic acid. After centrifugation (10,000 x *g*, 5 min), the supernatants were filtered through RC 58 0.2-im cellulose membranes (Bioanalytical Systems, West Lafayette, IN, USA). The mobile phase consisted of 0.05 mM citrate-phosphate buffer, pH 3.5, 0.1 mM sodium octyl sulfonate and 3.5% methanol. The flow rate was maintained at 1 ml/min. NA, 5-HT and their metabolites were quantified by peak height comparisons with standards run on the day of analysis [35].

Calculations and statistics

The data from behavioral tests (FST, TST) and neurochemical studies were analyzed by means of a one-way analysis of variance (ANOVA), followed when appropriate by Duncan's *post hoc* test. The results were considered statistically significant when $p < 0.05$.

The total rate of NA metabolism was assessed from the ratio of the final NA metabolite concentration, MHPG to NA concentration and expressed as the metabolism rate index: [MHPG]/[NA] × 100; the factor of NA re-uptake inhibition as the ratio: [NMN]/[MHPG] × 100 and the rate of NA release as the ratio: [NMN]/[NA] × 100. Analogously, the rate of 5-HT metabolism was expressed as the ratio: [5-HIAA]/[5HT] × 100. The indices were calculated using concentrations from individual tissue samples [30].

The results of locomotor activity experiments were evaluated by means of a one-way analysis of variance (ANOVA) for

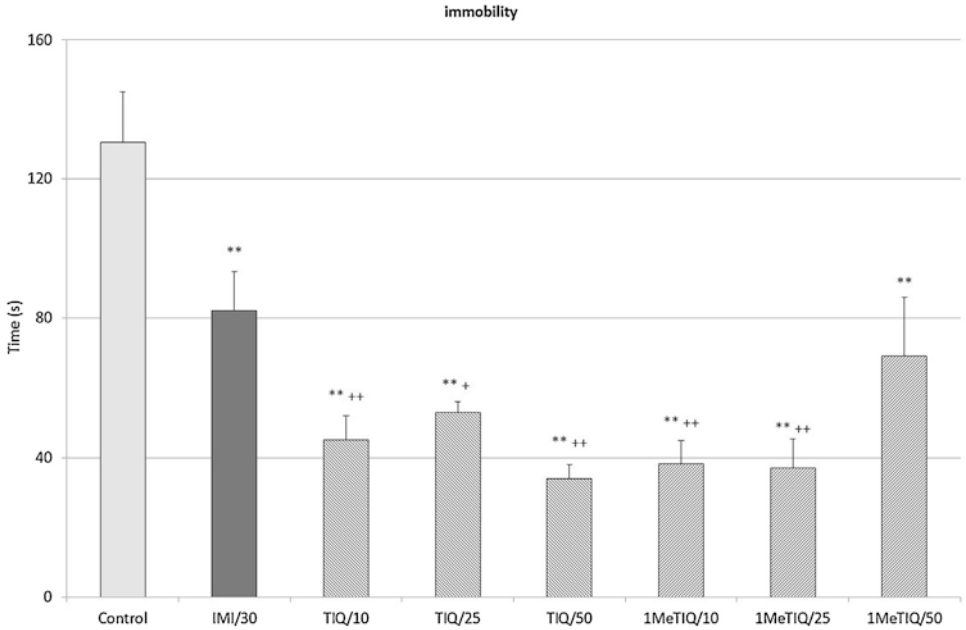

Fig. 2. *The effect of various doses of TIQ and 1MeTIQ in the FST in comparison with imipramine.* The mice received a single injection of saline (Control), imipramine (30 mg/kg), TIQ or 1MeTIQ (10, 25, 50 mg/kg) 1 h before the FST. The data are presented as the means ± SEM. The number of animals per group, n = 6-7. Statistical significance: $^{**}p < 0.01$ *vs.* control group; $^{+}p < 0.05$, $^{++}p < 0.01$ *vs.* imipramine-treated group; *post hoc* Duncan's test.

repeated measures, followed when appropriate by LSD *post hoc* test. The data were considered statistically significant when $p < 0.05$.

Results

Behavioral studies

The effect of various doses of TIQ and 1MeTIQ in the FST in comparison with imipramine (Fig. 2)

Acute treatment with all investigated substances: TIQ or 1MeTIQ (10, 25 and 50 mg/kg), or imipramine (30 mg/kg) significantly reduced the immobility time in the FST in mice ($F(7.41) = 10.72$, $p < 0.01$) (Fig. 2).

The effect of various doses of TIQ and 1MeTIQ in the TST in comparison with imipramine (Fig. 3)

The results of TST showed that imipramine (15 mg/kg; $p < 0.05$) and TIQ (25 mg/kg; $p < 0.01$) significantly decreased the immobility time in this test in mice ($F(5.42) = 8.01$), $p < 0.01$) (Fig. 3).

The effect of various doses of TIQ and 1MeTIQ on the locomotor activity in mice in comparison with imipramine (Fig. 4A and B)

The results showed that imipramine (30 mg/kg), TIQ or 1MeTIQ (10, 25 mg/kg) significantly decreased the motor activity in mice C57BL6J during the 60 min of locomotor activity test (Fig. 4A and B, respectively). The one-way ANOVA for repeated measures revealed a significant effect of Treatment (TIQ: $F(3.27) = 9.30$, $p < 0.01$; 1MeTIQ: $F(3.28) = 6.72$, $p < 0.01$), and Time (TIQ: $F(5.135) = 26.90$, $p < 0.01$; 1MeTIQ: $F(5.140) = 71.98$, $p < 0.01$). Also interaction of Treatment *vs.* Time was significant for both investigated substances $F(15.135) = 9.21$, $p < 0.01$ for TIQ and $F(15.140) = 3.31$, $p < 0.01$ for 1MeTIQ, respectively.

Biochemical ex vivo *studies*

The effect of various doses of TIQ and 1MeTIQ on the concentration of NA and its metabolites in mouse brain structures and on the rate of NA metabolism in the striatum (Table 1; Fig. 5A–C)

Substantia nigra. The one-way ANOVA demonstrated a significant effect of TIQs on the concentration of NA ($F(5.29) = 2.59$, $p \leq 0.05$) and its metabolites: NMN ($F(5.29) = 67.09$, $p < 0.01$) and MHPG ($F(5.29) = 22.18$, $p \leq 0.01$). The Duncan's *post hoc* test showed that TIQs administration caused a dose-dependent elevation of the

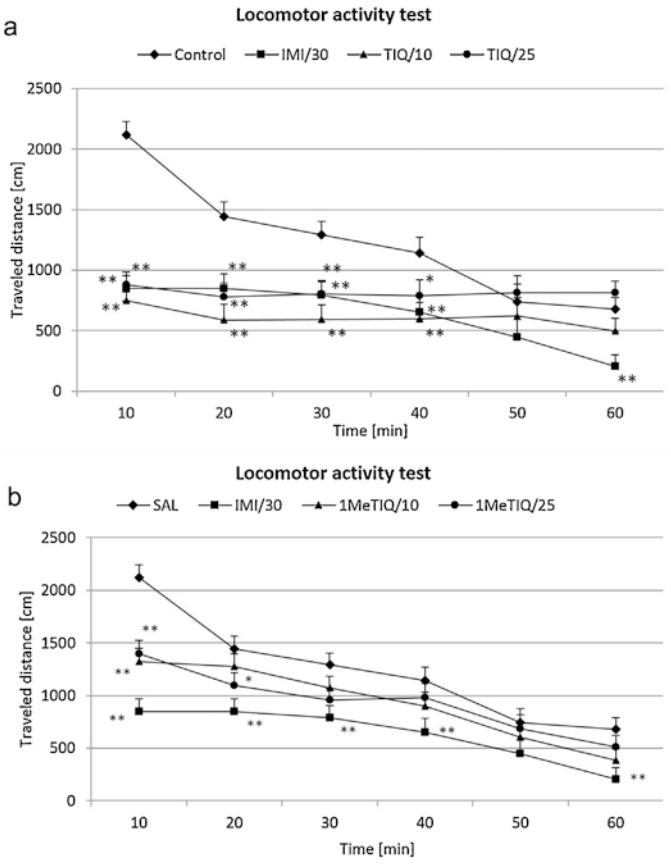

Fig. 4. *The effect of various doses of TIQ (A) and 1MeTIQ (B) on the locomotor activity in mice in comparison with imipramine.* The mice received a single injection of saline (Control), imipramine (30 mg/kg), TIQ or 1MeTIQ (10, 25 mg/kg) and 60 min later they were placed into Opto-M3 System cages for 60 min. The data are presented as the means ± SEM. The number of animals per group, n = 8. The data were analyzed by means of a one-way analysis of variance (ANOVA) for repeated measures, followed when appropriate by LSD *post hoc* test. Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* control group.

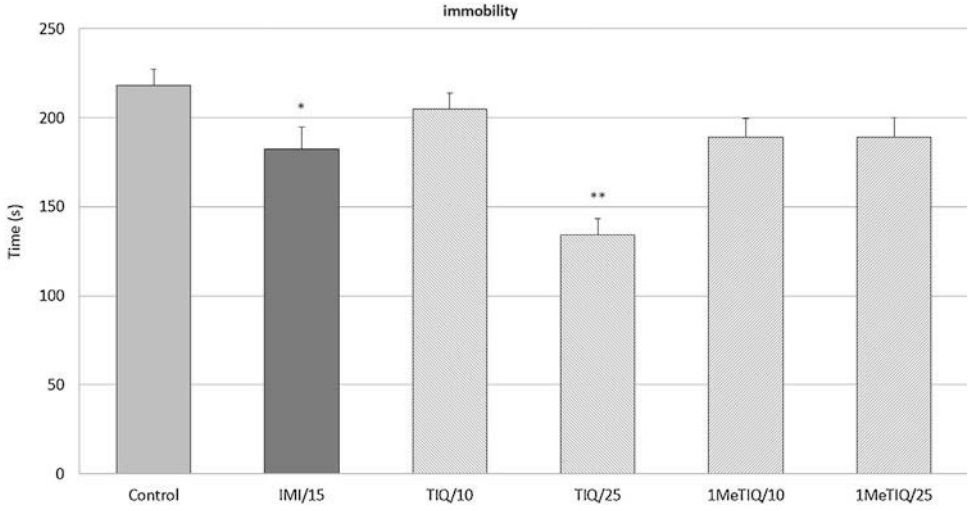

Fig. 3. *The effect of various doses of TIQ and 1MeTIQ in the TST in comparison with imipramine.* The mice received a single injection of saline (Control), imipramine (15 mg/kg), TIQ or 1MeTIQ (10, 25 mg/kg) 1 h before the TST. The immobility time of mice was measured during the behavioral test. The data are presented as the means ± SEM. The number of animals per group, n = 8. Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* control group; *post hoc* Duncan's test.

Table 1
The effect of various doses of TIQ and 1MeTIQ on the concentration of NA and its metabolites in comparison with imipramine in mice brain structures.

Treatment (mg/kg)	NA	NMN	MHPG
	(ng/g tissue)		
Substantia nigra			
Control	608 ± 41	31 ± 3.9	48 ± 4.2
IMI/30	704 ± 34	32 ± 2.5	39 ± 2.8
TIQ/25	780 ± 51*	84 ± 6.0^{**++}	92 ± 5.3^{**++}
TIQ/50	803 ± 65**	149 ± 7.4^{**++}	78 ± 4.2^{**++}
1MeTIQ/25	772 ± 37*	61 ± 3.6^{**++}	56 ± 5.1^{+}
1MeTIQ/50	789 ± 41*	101 ± 6.8^{**++}	49 ± 3.2
F	$F_{(5/29)} = 2.59$	$F_{(5/29)} = 67.09$	$F_{(5/29)} = 22.18$
	$p \leq 0.05$	$p < 0.01$	$p < 0.01$
Striatum			
Control	92 ± 14.7	13 ± 1.2	49 ± 2.8
IMI/30	89 ± 9.0	16 ± 1.9	29 ± 1.5**
TIQ/25	104 ± 8.7	47 ± 6.6^{**++}	55 ± 3.0^{++}
TIQ/50	123 ± 6.1	63 ± 3.0^{**++}	35 ± 0.7**
1MeTIQ/25	123 ± 15.7	21 ± 2.1	35 ± 5.2**
1MeTIQ/50	141 ± 13.0^{*+}	35 ± 2.2^{**++}	25 ± 1.1**
F	$F_{(5/30)} = 2.95$	$F_{(5/30)} = 34.43$	$F_{(5/30)} = 17.05$
	$p < 0.03$	$p < 0.01$	$p < 0.01$
Frontal cortex			
Control	284 ± 11.8	30 ± 2.0	41 ± 1.1
IMI/30	304 ± 6.7	41 ± 1.8	28 ± 1.0**
TIQ/25	199 ± 6.7^{**++}	159 ± 9.4^{**++}	77 ± 2.7^{**++}
TIQ/50	199 ± 10.9^{**++}	221 ± 8.8^{**++}	49 ± 1.9^{*++}
1MeTIQ/25	310 ± 7.9	56 ± 3.5**	44 ± 3.3^{++}
1MeTIQ/50	323 ± 5.5**	93 ± 3.0^{**++}	42 ± 1.2^{++}
F	$F_{(5/30)} = 42.80$	$F_{(5/30)} = 175.35$	$F_{(5/30)} = 62.24$
	$p < 0.01$	$p < 0.01$	$p < 0.01$
Hypothalamus			
Control	1431 ± 87	25 ± 1.5	58 ± 6.0
IMI/30	1476 ± 86	35 ± 1.3	39 ± 2.5**
TIQ/25	1464 ± 60	101 ± 6.8^{**++}	80 ± 4.0^{**++}
TIQ/50	1400 ± 60	220 ± 12.0^{**++}	66 ± 1.1^{++}
1MeTIQ/25	1384 ± 47	46 ± 3.1*	57 ± 7.8^{++}
1MeTIQ/50	1403 ± 39	105 ± 7.6^{**++}	48 ± 4.7
F	$F_{(5/30)} = 0.31$	$F_{(5/30)} = 119.94$	$F_{(5/30)} = 8.40$
	ns	$p < 0.01$	$p < 0.01$

TIQ and 1MeTIQ were administrated once in two various doses: 25 and 50 mg/kg. Imipramine was administrated in a dose 30 mg/kg. Control group received saline. Animals were decapitated 60 min after injection, immediately after the FST. The concentrations of NA and its metabolites were measured in ng/g wet tissue. The data are the means ± SEM. The results were analyzed by means of one-way ANOVA analysis of variance, followed when appropriate by *post hoc* Duncan's test. Statistical significance: $^{*}p < 0.01$, $^{**}p < 0.05$ *vs.* control group; $^{+}p < 0.01$, $^{++}p < 0.05$ *vs.* imipramine-treated group.

level of NA (up to 130%) and its metabolites: NMN (up to 480%, $p < 0.01$) and MHPG (up to 190%, $p < 0.01$) (Table 1).

Striatum. The statistical analysis demonstrated a significant effect of TIQs and imipramine on the NA ($F(5.30) = 2.95$, $p < 0.03$), NMN ($F(5.30) = 34.43$, $p < 0.01$) and MHPG ($F(5.30) = 17.05$, $p < 0.01$) concentration. The Duncan's *post hoc* test showed that 1MeTIQ (50 mg/kg) significantly increased the NA concentration (up to 153%, $p < 0.03$). Additionally, TIQ (25, 50 mg/kg) and 1MeTIQ (50 mg/kg) significantly elevated the level of NMN (about 260, 385 and 170%, $p < 0.01$, respectively). Imipramine (30 mg/kg), TIQ (50 mg/kg) and both doses of 1MeTIQ significantly decreased the final NA metabolite, MHPG concentration ($p < 0.01$) (Table 1). The one-way ANOVA revealed a significant effect of the investigated compounds on the NA metabolism. The NA release index was significantly increased ($F(5.30) = 20.84$, $p < 0.01$) after TIQ (25, 50 mg/kg) administration (Fig. 5A). Additionally, the index of NA re-uptake inhibition was dose-dependently elevated by TIQs ($F(5.30) = 31.98$, $p < 0.01$) (Fig. 5B).The Duncan's *post hoc* test indicated also that imipramine (30 mg/kg), TIQ (50 mg/kg) and 1MeTIQ (25, 50 mg/kg) significantly decreased the rate NA metabolism ($F(5.30) = 11.49$, $p < 0.01$) (Fig. 5C).

Frontal cortex. The one-way ANOVA showed a significant effect of treatment with the investigated substances on the NA ($F(5.30) = 42.80$, $p < 0.01$) and its metabolites, NMN ($F(5.30) = 175.35$, $p < 0.01$) and MHPG ($F(5.30) = 62.24$, $p < 0.01$) concentration. The Duncan's *post hoc* test revealed that TIQ significantly decreased the level of NA (about 30%, $p < 0.01$) while 1MeTIQ produced the opposite effect (up to 110%, $p < 0.01$). On the other hand, TIQs administration significantly elevated the NMN concentration (approx. 530%, $p < 0.01$–TIQ; 150%, $p < 0.01$–1MeTIQ). Imipramine produced a significant decrease in the MHPG level (approx. 30%, $p < 0.01$) while TIQ (25, 50 mg/kg) showed an opposite effect (up to 90%, $p < 0.01$ and 20%, $p < 0.05$, respectively) (Table 1).

Hypothalamus. The one-way ANOVA demonstrated no effect of the investigated substances on the NA concentration ($F(5.30) = 0.31$, ns). The same statistical test indicated a significant effect of TIQ and its methyl derivative, 1MeTIQ on the level of metabolites of this monoamine: NMN ($F(5.30) = 119.94$, $p < 0.01$) and MHPG ($F(5.30) = 8.40$, $p < 0.01$). The Duncan's *post hoc* test demonstrated that an acute dose of TIQ or 1MeTIQ (25, 50 mg/kg) strongly increased the level of NMN (up to approx. 200–880%, $p < 0.01$). Additionally, TIQ significantly elevated the level of the final NA metabolite, MHPG (up to 140%, $p < 0.01$) (Table 1).

The effect of various doses TIQ and 1MeTIQ on the concentration of 5-HT and its metabolite in mouse brain structures and on the rate of 5-HT metabolism in the striatum (Table 2; Fig. 6)

Substantia nigra. The one-way ANOVA showed no significant effect of TIQs and imipramine on the 5-HT ($F(5.29) = 0.84$, ns) concentration in the mouse substantia nigra. The statistical analysis revealed also the impact of the tested substances on the level of 5-HIAA ($F(5.29) = 8.11$, $p < 0.01$). The Duncan's *post hoc* test demonstrated that TIQ (50 mg/kg) and 1MeTIQ (25, 50 mg/kg), similarly to imipramine, decreased the 5-HIAA concentration (about 30%, $p < 0.01$–TIQ; 20%, $p < 0.05$ and 40%, $p < 0.01$–1MeTIQ; 20%, $p < 0.01$–IMI, respectively) (Table 2).

Striatum. The one-way ANOVA demonstrated no effect of treatment with TIQ, 1MeTIQ and imipramine on the 5-HT concentration ($F(5.30) = 0.69$, ns). The same statistical analysis indicated a strong effect of the investigated compounds on the level of 5-HIAA ($F(5.30) = 10.59$, $p < 0.01$). The Duncan's *post hoc* test showed that TIQs dose-dependently decreased the 5-HIAA concentration (approx. 13%, $p < 0.05$ and 30%, $p < 0.01$–TIQ; 20 and 30%, $p < 0.01$–1MeTIQ, respectively) (Table 2). The one-way ANOVA revealed a significant effect of TIQs and imipramine injection on the rate of 5-HT metabolism ($F(5.30) = 16.01$, $p < 0.01$). The *post hoc* analysis showed that imipramine, TIQ and 1MeTIQ dose-dependently reduced the rate of 5-HT metabolism in the striatum (Fig. 6).

Frontal cortex. The one-way ANOVA demonstrated a significant effect of the investigated substances on the 5-HT ($F(5.30) = 4.09$, $p < 0.01$) and 5-HIAA ($F(5.30) = 27.02$, $p < 0.01$) concentration. The Duncan's *post hoc* test revealed that TIQ and 1MeTIQ (25, 50 mg/kg) significantly increased the level of 5-HT (approx. 15%, $p < 0.01$). On the other hand, TIQs or imipramine (30 mg/kg) administration decreased the 5-HIAA concentration (approx. 30%, $p < 0.01$) (Table 2).

Hypothalamus. As shown in Table 2, TIQs and the reference drug, imipramine caused a significant effect on the 5-HT ($F(5.30) = 10.14$, $p < 0.01$) and 5-HIAA ($F(5.30) = 23.66$, $p < 0.01$) concentration. The

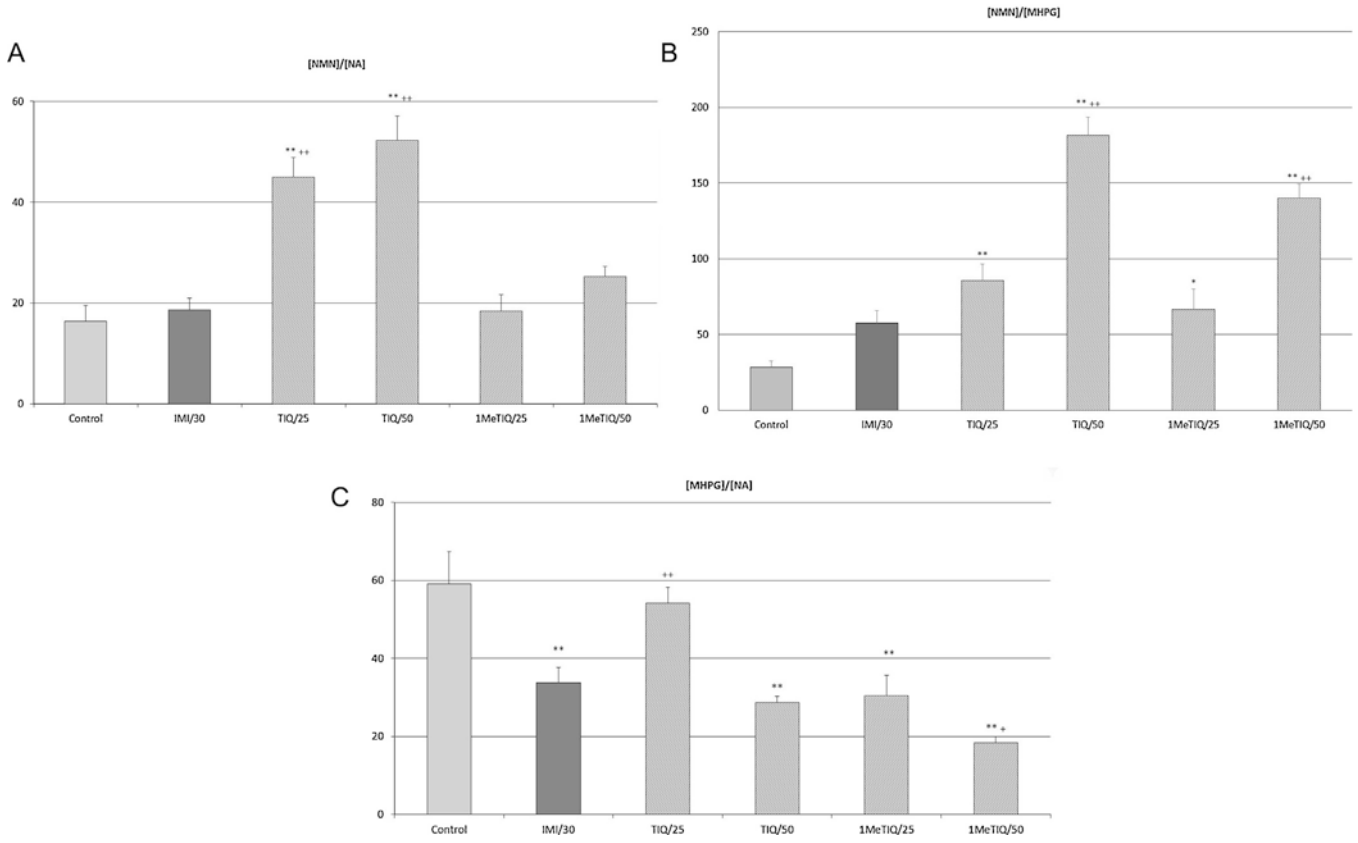

Fig. 5. *The effect of various doses of TIQ and 1MeTIQ, compared with imipramine, on the rate of NA metabolism in the mouse striatum.* The NA release index was expressed as the ratio: [NMN]/[NA] × 100 (A), the index of NA re-uptake inhibition was expressed as [NMN]/[MHPG] × 100 (B) and the rate of NA metabolism was calculated as the ratio: [MHPG]/[NA] × 100 (C). The data are presented as the means ± SEM. The indices were calculated using the concentrations of individual tissue samples (n = 6). The results were analyzed by means of a one-way analysis of variance (ANOVA), followed when appropriate, by *post hoc* Duncan's test. Statistical significance: $^{*}p < 0.01$, $^{**}p < 0.05$ *vs.* control group; $^{+}p < 0.01$, $^{++}p < 0.05$ *vs.* imipramine-treated group.

Duncan's *post hoc* test showed elevation of the 5-HT level (about 20%, $p < 0.01$) after TIQ (25, 50 mg/kg) administration. The same analysis demonstrated also that TIQ and 1MeTIQ administration caused a dose-dependent reduction of the 5-HIAA level (approx. 19% at a lower dose and 30% at a higher dose, $p < 0.01$). Similar effect was observed after imipramine administration ($p < 0.01$) (Table 2).

Discussion

In the present study, we demonstrate, to our knowledge for the first time, antidepressant-like potential of both investigated compounds TIQ and 1MeTIQ in behavioral tests in mice. The behavioral despair tests: FST and TST are widely used as useful models for probing the pathological mechanism of depression and for evaluation of antidepressant drugs [38,41,42]. These tests are also well-established experimental models for screening new potent antidepressant drugs [9]. In the FST and TST, animals under stress conditions from which they cannot escape, become immobile after an initial period of struggling. This characteristic behavior scored in both tests and termed immobility, reflecting a behavioral state of despair (learned helplessness), resembles the state of mental depression which can be reduced by antidepressant drugs [38,41].

In the present study, we investigated the effects of TIQ and its methyl derivative, 1MeTIQ administration on the duration of immobility in the FST and TST in C57BL6J mice. Indeed, the sensitivity of the FST to an impressively broad range of antidepressant drugs is one of the most important features supporting its primary use as a screen in antidepressant discovery research [36,38].

The results presented here show that these exo/endogenous amines administrated systemically in various doses to mice are effective in producing a significant antidepressant-like activity. They decreased immobility time in the FST and TST similarly to a classical antidepressant, imipramine (Figs. 2 and 3). Imipramine is a frequently used antidepressant drug in the treatment of major depression which moderates mainly 5-HT and NA reuptake inhibition [43–48]. In view of this, animal behavior in mice was evaluated for specific involvement of serotonergic or noradrenergic pathways in the antidepressant activity. We observed that both investigated substances acted with the potency comparable to that of the prototypic antidepressant drug – imipramine when assessed in the FST, TST and locomotor activity test (Figs. 2, 3, 4A and B).

It is important to demonstrate that antidepressant-like effect in the FST and TST was distinguished from psychostimulants because psychostimulants cause marked motor stimulation in contrast to antidepressants, which do not. In order to determine whether TIQ and 1MeTIQ actually possess an antidepressant-like activity, we tested the locomotion counts to exclude the psychostimulating effects after administration of these compounds. It should be taken into account that the doses of TIQ and 1MeTIQ (10 and 25 mg/kg) that decreased the immobility time in the FST and TST produced a reduction in the locomotor activity test. Indeed, the tricyclic antidepressant – imipramine, exhibits an antidepressant-like activity in the FST or TST and similarly to investigated compounds (TIQ and 1MeTIQ) produced a sedative effect in the locomotor activity test in mice (Figs. 2, 3, 4A and B).

Table 2
The effect of various doses of TIQ and 1MeTIQ on the concentration of 5-HT and its metabolite in comparison with imipramine in mice brain structures.

Treatment (mg/kg)	5-HT (ng/g tissue)	5-HIAA
Substantia nigra		
Control	984 ± 61	319 ± 14
IMI/30	995 ± 31	254 ± 13*
TIQ/25	1099 ± 66	277 ± 22
TIQ/50	1140 ± 70	213 ± 15**
1MeTIQ/25	1033 ± 71	261 ± 8*
1MeTIQ/50	1030 ± 78	190 ± 20^{**++}
F	$F_{(5/29)} = 0.84$ ns	$F_{(5/29)} = 8.11$ $p < 0.01$
Striatum		
Control	513 ± 16	349 ± 16.7
IMI/30	525 ± 11	270 ± 3.9**
TIQ/25	529 ± 13	305 ± 8.0*
TIQ/50	528 ± 29	251 ± 10.8**
1MeTIQ/25	488 ± 20	284 ± 16.8**
1MeTIQ/50	526 ± 19	237 ± 12.6**
F	$F_{(5/30)} = 0.69$ ns	$F_{(5/30)} = 10.59$ $p < 0.01$
Frontal cortex		
Control	458 ± 12	243 ± 9.5
IMI/30	488 ± 20	177 ± 4.1**
TIQ/25	531 ± 10**	198 ± 3.7^{**+}
TIQ/50	511 ± 14**	147 ± 2.8^{**+}
1MeTIQ/25	524 ± 14**	196 ± 9.5**
1MeTIQ/50	525 ± 9**	152 ± 7.6^{**+}
F	$F_{(5/30)} = 4.09$ $p < 0.01$	$F_{(5/30)} = 27.02$ $p < 0.01$
Hypothalamus		
Control	1204 ± 20	314 ± 4.6
IMI/30	1301 ± 51	228 ± 8.1**
TIQ/25	1456 ± 16^{**++}	276 ± 4.4^{**++}
TIQ/50	1444 ± 29^{**++}	222 ± 2.5**
1MeTIQ/25	1271 ± 36	267 ± 15.2^{**++}
1MeTIQ/50	1292 ± 23	209 ± 7.7**
F	$F_{(5/30)} = 10.14$ $p < 0.01$	$F_{(5/30)} = 23.66$ $p < 0.01$

TIQ and 1MeTIQ were administrated once in two various doses: 25 and 50 mg/kg. Imipramine was administrated in a dose 30 mg/kg. Control group received saline. Animals were decapitated 60 min after injection, immediately after the FST. The concentrations of 5-HT and its metabolite were measured in ng/g wet tissue. The data are the means ± SEM. The results were analyzed by means of one-way ANOVA analysis of variance, followed when appropriate by *post hoc* Duncan's test. Statistical significance: $^{*}p < 0.01$, $^{**}p < 0.05$ *vs.* control group; $^{+}p < 0.01$, $^{++}p < 0.05$ *vs.* imipramine-treated group.

Additionally to the behavioral tests, the effects of TIQs on the levels of monoamines and their metabolites in the mouse brain were examined. The results of neurochemical analysis perfectly characterize and differentiate the impact of TIQ, its methyl derivative 1MeTIQ and imipramine on the activity of noradrenergic system through the presented ratios. TIQ reveals a significant and dose-dependent impact on the NA release measured by the ratio [NMN/NA]. Our *in vivo* microdialysis study have demonstrated that TIQ administered systemically to rat in a dose 50 mg/kg produced a powerful release of NA (about 1200% of control) in the frontal cortex (data not shown). It seems that the effect may be similar in the C57BL6J mice investigated in the present paper. Such mechanism of action of TIQ may suggest antagonism to presynaptic alpha2-adrenoceptor. This effect was not observed in such intensity after 1MeTIQ or imipramine administration (Table 1, Fig. 5A). What is more, TIQ and 1MeTIQ produce a significant and dose-dependent inhibition of the NA reuptake measured as an index of [NMN]/[MHPG]. Imipramine works in the same direction but a little bit weaker (Table 1, Fig. 5B). At the same time, all investigated substances as NA reuptake inhibitors significantly reduced the total rate of NA metabolism expressed as the ratio [MHPG]/[NA] through the feedback mechanism (Table 1, Fig. 5C).

Further, we suggest that both noradrenergic postsynaptic alpha1 and alpha2 receptors are implicated in the mechanism of action of TIQs in the FST and TST. How it was previously demonstrated the antidepressant-like effect of desipramine in FST was prevented by the pretreatment of mice with prazosin [49]. What is more, phenylephrine is a postsynaptic alpha1-adrenoceptor agonist that was shown to reduce the duration of immobility time in the FST [50]. In addition, the repeated treatment with antidepressants produced an increase in the density of alpha1-adrenergic receptors [51]. Then, the alpha2-agonist, clonidine also reduced the immobility time in FST and potentiates the effects antidepressant drugs in mice and rats [52,55]. However, imidazoline (selective I_2 ligands) did not show antidepressant-like activity in FST in mice [53].

In the serotonergic pathway, the inhibition of striatal 5-HT metabolism rate [5-HIAA]/[5-HT] caused by TIQs and imipramine is reinforcement with the serotonergic system activation through the 5-HT reuptake inhibition as well. TIQ and 1MeTIQ produced a significant elevation of 5-HT concentration in the brain structures (frontal cortex and hypothalamus) with simultaneous reduction of

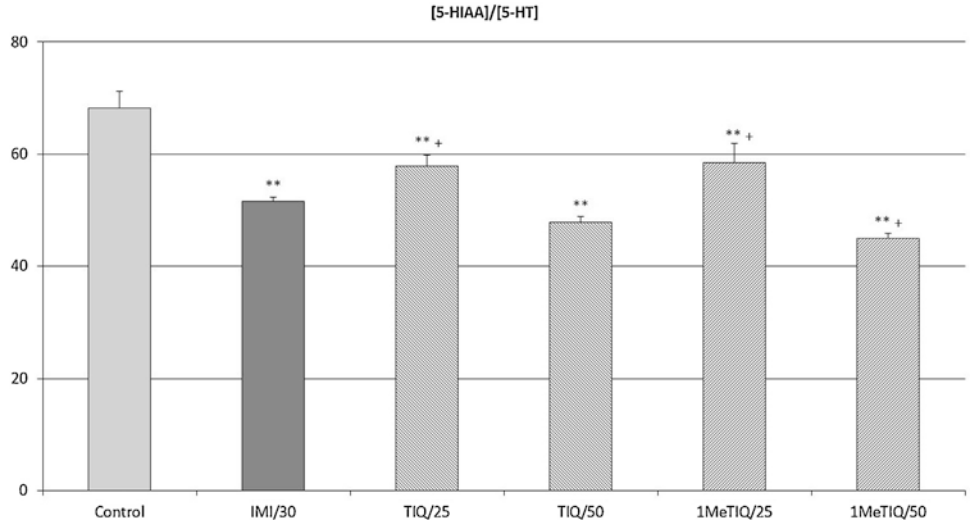

Fig. 6. *The effect of various doses of TIQ and 1MeTIQ, compared with imipramine, on the rate of 5-HT metabolism in the mouse striatum.* It was expressed as the ratio: [5-HIAA]/[5-HT] × 100. The data are presented as the means ± SEM. The indices were calculated using the concentrations of individual tissue samples (n = 6). The results were analyzed by means of a one-way analysis of variance (ANOVA), followed when appropriate, by *post hoc* Duncan's test. Statistical significance: $^{*}p < 0.01$, $^{**}p < 0.05$ *vs.* control group; $^{+}p < 0.01$, *vs.* imipramine-treated group.

its metabolite, 5-HIAA. The effect of imipramine was weaker and limited to the essential fall of 5-HIAA concentration (Table 2, Fig. 6). Imipramine, as well-known tricyclic antidepressant acts as an inhibitor of both 5-HT and NA reuptake [54]. In the case of TIQ and 1MeTIQ the intensification of 5-HT transmission is connected both with its reuptake inhibition [55] as well as with the inhibition of MAO degradation [34]. It is important to mention that antidepressant-like activity of TIQ and 1MeTIQ demonstrated in the present paper in C57BL6J mice was also observed in the Wistar rats, and the data from behavioral and neurochemical experiments are compatible in these two species of rodents [35].

Summing up, this study provides evidences that TIQ and 1MeTIQ show a potent antidepressant-like activity and they might possess an adrenergic and serotonergic component of pharmacological activity. Their mechanism of antidepressant-like action is similar but at least wider compared with that of imipramine. TIQ and 1MeTIQ easily penetrate into the brain after peripheral administration and their interesting action on the monoaminergic neurotransmitter pathways may be beneficial from the clinical point of view thus, they are good candidates for antidepressants with helpful antioxidant and neuroprotective activity [14,15,17,18].

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References

[1] Cantello R, Aguggia M, Gilli M, Delsedime M, Cutin IC, Riccio A, et al. Major depression in Parkinson’s disease and the mood response to intravenous methylphenidate: possible role of the hedonic dopamine synapse. J Neurol Neurosurg Psychiatry 1989;52:724–31.
[2] Chan-Palay V, Asan E. Alterations in catecholamine neurons of the locus coeruleus in senile dementia of Alzheimer type and in Parkinson’s disease with and without dementia and depression. J Comp Neurol 1989;287:373–92.
[3] Colpaert FC. Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rats. Neuropharmacology 1987;26:1431–40.
[4] Antkiewicz-Michaluk L. Action of antidepressant neuroleptics chlorprothixene and levomepromazine of the central noradrenergic system comparison with other antidepressants. Pol J Pharmacol Pharm 1985;37:667–77.
[5] Maj J, Przegaliński E, Mogilnicka E. Hypotheses concerning the mechanism of action of antidepressant drugs. Rev Physiol Biochem Pharmacol 1984;100:1–74.
[6] Sulser F. New perspectives on the molecular pharmacology of affective disorders. Eur Arch Psych Neurol 1989;238:231–9.
[7] Richelson E. Pharmacology of antidepressants —characteristics of the ideal drug. Mayo Clinic Proc 1994;69:1069–81.
[8] Vetulani J, Nalepa I. Antidepressants: past, present and future. Eur J Pharmacol 2000;405:351–63.
[9] Bourin M. Is it possible to predict the activity of a new antidepressant in animals with simple psychopharmacological test. Fund Clin Pharmacol 1990;4:49–64.
[10] Antkiewicz-Michaluk L, Michaluk J, Romańska I, Vetulani J. Effect of repetitive electroconvulsive treatment on sensitivity to pain and on [3H]-nitrendipine binding sites in cortical and hippocampal membranes. Psychopharmacology 1990;101:240–3.
[11] Antkiewicz-Michaluk L, Romańska I, Michaluk J, Vetulani J. Role of calcium channels in effects of antidepressant drugs on responsiveness to pain. Psychopharmacology 1991;105:269–74.
[12] Maj J, Górka Z, Melzacka M, Rawłów A, Pilc A. Chronic treatment with imipramine: further functional evidence for the enhanced noradrenaline transmission in flexor reflex activity. Naunyn-Schmied Arch Pharmacol 1983;322:256–60.
[13] Sulser F. The role of CREB and other transcription factors in the pharmacotherapy and etiology of depression. Ann Med 2002;34:348–56.
[14] Rommelspacher H, Susilo R. Tetrahydroisoquinolines and beta-carbolines: putative natural substances in plants and mammals. Prog Drug Res 1985;29:415–59.
[15] Makino Y, Ohta S, Tachikawa O, Hirobe M. Presence of tetrahydroisoquinoline and 1-methyl-tetrahydroisoquinoline in foods: compounds related to Parkinson’s disease. Life Sci 1988;43:373–8.
[16] Niwa T, Yoshizumi H, Tatematsu A, Matsuura S, Nagatsu T. Presence of tetrahydroisoquinoline, a parkinsonism-related compound, in foods. J Chromatogr 1989;493:347–52.
[17] McNaught KS, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, et al. Isoquinoline derivatives as endogenous neurotoxins in the etiology of Parkinson's disease. Biochem Pharmacol 1998;56:921–33.
[18] Yamakawa T, Kotake Y, Fujitani M, Shintani H, Makino Y, Ohta S. Regional distribution of parkinsonism-preventing endogenous tetrahydroisoquinoline derivatives and an endogenous parkinsonism-preventing substance-synthesizing enzyme in monkey brain. Neurosci Lett 1999;276:67–70.
[19] Nagatsu T. Isoquinoline neurotoxins in the brain and Parkinson's disease. Neurosci Res 1997;29:99–111.
[20] Naoi M, Matsuura S, Parvez H, Takahashi T, Hirata Y, Minami M, et al. Oxidation of N-methyl-1,2,3,4-tetrahydroisoquinoline into the N-methyl-isoquinolinium ion by monoamine oxidase. J Neurochem 1989;52:653–5.
[21] Yamakawa T, Ohta S. Biosynthesis of a parkinsonism-preventing substance, 1-methyl-1,2,3,4-tetrahydroisoquinoline, is inhibited by parkinsonism-inducing compounds in rat brain mitochondrial fraction. Neurosci Lett 1999;259:157–60.
[22] Yamakawa T, Ohta S. Isolation of 1-methyl-1,2,3,4-tetrahydroisoquinoline-synthesining enzyme from rat brain: a possible Parkinson’s disease-preventing enzyme. Biochem Biophys Res Commun 1997;236:676–81.
[23] Naoi M, Maruyama W, Nagy GM. Dopamine-derived salsolinol derivatives as endogenous monoamine oxidase inhibitors: occurrence, metabolism and function in human brains. Neurotoxicology 2004;25:193–204.
[24] Lorenc-Koci E, Wójcikowski J, Kot M, Haduch A. Disposition of 1,2,3,4-tetrahydroisoquinoline in the brain of male Wistar and Dark Agouti rats. Brain Res 2004;996:168–79.
[25] Lorenc-Koci E, Antkiewicz-Michaluk L, Wardas J, Zapała M. Effect of 1,2,3,4-tetrahydroisoquinoline administration under conditions of CYP2D inhibition on dopamine metabolism, level of tyrosine hydroxylase proteinand the binding of [3H]GBR12,935 to dopamine transporter in the rat nigrostriatal, dopaminergic system. Brain Res 2004;1009:67–81.
[26] Antkiewicz-Michaluk L, Karolewicz B, Romańska I, Michaluk J, Bojarski A, Vetulani J. 1-Methyl-1,2,3,4-tetrahydroisoquinoline protects against rotenone-induced mortality and biochemical changes in rat brain. Eur J Pharmacol 2003;466:263–9.
[27] Antkiewicz-Michaluk L, Wardas J, Michaluk J, Romańska I, Bojarski A, Vetulani J. Protective effect of 1-methyl-1,2,3,4-tetrahydroisoquinoline against dopaminergic neurodegeneration in the extrapyramidal structures produced by intracerebral injection of rotenone. Int J Neuropsychopharmacol 2004;7:155–63.
[28] Lorenc-Koci E, Sokołowska M, Kwiecień I, Włodek L. Treatment with 1,2,3,4-tetrahydroisoquinoline affects the levels of nitric oxide, S-nitrosothiols, glutathione and the enzymatic activity of gamma-glutamyl transpeptidase in the dopaminergic structures of rat brain. Brain Res 2005;1049:133–46.
[29] Antkiewicz-Michaluk L, Łazarewicz JW, Patsenka A, Kajta M, Ziemińska E, Salińska E, et al. The mechanism of 1,2,3,4-tetrahydroisoquinolines neuroprotection: the importance of free radicals scavenging properties and inhibition of glutamate-induced excitotoxicity. J Neurochem 2006;97:846–56.
[30] Antkiewicz-Michaluk L, Michaluk J, Mokrosz M, Romanska I, Lorenc-Koci E, Ohta S, et al. Different action on dopamine catabolic pathways of two endogenous 1,2,3,4-tetrahydroisoquinolines with similar antidopaminergic properties. J Neurochem 2001;78:100–8.
[31] Antkiewicz-Michaluk L, Filip M, Michaluk J, Romańska I. An endogenous neuroprotectant substance, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ), prevents the behavioral and neurochemical effects of cocaine reinstatement in drug-dependent rats. J Neural Transm 2007;114:307–17.
[32] Maruyama W, Nakahara D, Dostert P, Takahashi A. Naturally-occuring isoquinolines perturb monoamine metabolism in the brain: studied by in vivo microdialysis. J Neural Transm Gen Sect 1993;94:91–102.
[33] Lorenc-Koci E, Śmiałowska M, Antkiewicz-Michaluk L, Gołembiowska K, Bajkowska M, Wolfarth S. Effect of acute and chronic administration of 1,2,3,4-tetrahydroisoquinoline on muscle tone, metabolism of dopamine in the striatum and tyrosine hydroxylase immunocytochemistry in the substantia nigra, in rats. Neuroscience 2000;95:1049–59.
[34] Patsenka A, Antkiewicz-Michaluk L. Inhibition of rodent brain monoamine oxidase and tyrosine hydroxylase by endogenous compounds —1,2,3,4-tetrahydroisoquinoline alkaloids. Pol J Pharmacol 2004;56:727–34.
[35] Możdżeń E, Papp M, Gruca P, Wąsik A, Romańska I, Michaluk J, et al. 1,2,3,4-Tetrahydroisoquinoline produces an antidepressant-like effect in the forced

swim test and chronic mild stress model of depression in the rat: neurochemical correlates. Eur J Pharmacol 2014;729:107–15.
[36] Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379–91.
[37] Kitamura Y, Kitagawa K, Kimoto S, Sagara H, Shibata K, Kawasaki H, et al. Selegilin exerts antidepressant-like effects during the forced swim test in adrenocorticotropic hormone-treated rats. J Pharmacol Sci 2008;106:639–44.
[38] Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977;229:327–36.
[39] Zomkowski AD, Santos AR, Rodrigues AL. Evidence for the involvement of the opioid system in the agmatine antidepressant-like effect in the forced swimming test. Neurosci Lett 2005;381:279–83.
[40] Zhou D, Jin H, Lin HB, Yang X-M, Cheng YF, Deng FJ, et al. Antidepressant effect of the extracts from Fructus Akebiae. Pharmacol Biochem Behav 2010;94:488–95.
[41] Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl) 1985;85:367–70.
[42] Wang YM, Kong LD, Chen YM. Behavioural and biochemical effects of fractions prepared fromBanxia Houpudecoction in depression models in mice. Phytother Res 2005;19:526–9.
[43] Javaid JI, Perel JM, Davis JM. Inhibition of biogenic amines uptake by imipramine, desipramine, 2 OH-imipramine and 2 OH-desipramine in rat brain. Life Sci 1979;24:21–8.
[44] Deakin JF. Depression and 5-HT. Int Clin Psychopharmacol 1991;6:23–8.
[45] Borsini F. Role of the serotonergic system in the forced swimming test. Neurosci Biobehav Rev 1995;19:377–95.
[46] Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 1995;121:66–72.
[47] Lenders JWM, Eisenhofer G, Abeling NGGM, Berger W, Murphy DL, Konings H, et al. Specific genetic deficiencies of the A and B isoenzymes of monoamine oxidase are characterized by distinct neurochemical and clinical phenotypes. J Clin Invest 1996;97:1010–9.
[48] Redrobe JP, Bourin M. Partial role of 5-HT_3, and 5-HT_2, receptors in the activity of antidepressants in the mouse forced swimming test. Eur J Pharmacol 1997;325:129–35.
[49] Danysz W, Kostowski W, Kozak W, Hauptmann M. On the role of noradrenergic neurotransmission in the action of desipramine and amitryptyline in animal models of depression. Pol J Pharmacol Pharm 1986;38:285–98.
[50] Kitada Y, Miyauchi T, Kanazawa Y, Nakamichi H, Satoh S. Involvement of α −and β_1-adrenergic mechanisms in the immobility-reducing action of desipramine in the forced swimming test. Neuropharmacology 1983;22:1055–60.
[51] Dziedzicka-Wasylewska M, Faron-Górecka A, Rogoz Z, Solich J. The effect of combined treatment with imipramine and amantadine on the behavioral reactivity of central α_1-adrenergic system in rats. Behav Pharmacol 2004;15:159–65.
[52] Malinge M, Bourin M, Colombel MC, Larousse C. Additive effects of clonidine and antidepressant drugs in the mouse forced-swimming test. Psychopharmacology 1988;96:104–9.
[53] O'Neill MF, Osborne DJ, Woodhouse SM, Conway MW. Selective imidazoline I_2 ligands do not show antidepressant-like activity in the forced swim test in mice. J Psychopharmacol 2001;15:18–22.
[54] Grunewald GL, Reitz TJ, Ruth JA, Vollmer S, Eiden LE, Rutledge CO. Inhibition of neuronal uptake of ^{3}H-biogenic amines into rat cerebral cortex by partially and fully saturated derivatives of imipramine and desipramine. The importance of the aromatic ring in adrenergic amines −part 3. Biochem Pharmacol 1979;28:417–21.
[55] Patsenka A, Michaluk J, Antkiewicz-Michaluk L. Tetrahydroisoquinoline alkaloids as endogenous inhibitors of brain monoamine oxidase, tyrosine hydroxylase and uptake of monoamines: *in vitro* study. 13th International Symposium on Molecular and Physiological Aspects of Regulatory Processes of the Organism. . p. 344 Abstracts.