DOI 10.1007/s11055-019-00761-9
Neuroscience and Behavioral Physiology, Vol. 49, No. 4, May, 2019

Acute Antidepressant Effects of Derivatives of 3-Hydroxypyridine and Succinic Acid in Experiments on Rats

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 103, No. 7, pp. 755–767, July, 2017. Original article submitted April 12, 2017. Revised version received May 18, 2017.

We report here a comparative analysis of the acute antidepressant actions of derivatives of 3-hydroxypyridine and succinic acid (emoxypine, Reamberin, and Mexidol) developing 30–45 min after single drug doses. The acute antidepressant effects of emoxypine, Reamberin, and Mexidol were assessed from the dynamics of reductions in the duration of "behavioral despair" in rats during 15-min sessions of forced swimming starting 30 min after drug administration. The stimulatory or sedative activities of the study drugs were also assessed, in terms of their influences on behavior in animals in the open field test. All derivatives of 3-hydroxypyridine and succinic acid studied here had acute antidepressant effects. The rate of onset of this effect and the presence of stimulatory or sedative activities depended on the characteristics of the chemical structures of the derivatives of 3-hydroxypyridine and succinic acid. The 3-hydroxypyridine-only derivative (emoxypine) was less active than the succinic acid derivatives (Reamberin and Mexidol) in terms of the rate of onset of the antidepressant effect and had stimulatory actions after single subantidepressant doses. Reamberin and Mexidol had acute antidepressant effects on the background of concomitant sedative effects.

Keywords: derivatives of 3-hydroxypyridine and succinic acid, acute antidepressant effects, stimulatory action, sedative action.

Depression is among the commonest mental disorders, occupying one of the leading positions in the overall structure of disease; it is a global medical-social problem of the modern age [15, 19]. Significant economic losses due to work incapacity because of depression, along with adverse effects on mental wellbeing and quality of life, determine the high demand for antidepressants on the pharmaceutical market [15, 19, 22]. Current antidepressants, like their historical prototypes (iproniazid and imipramine) are characterized by a stereotypical mechanism of action directed to compensating for a deficit in catecholaminergic and/or serotoninergic neurotransmission in synapses in the brain [22]. For more than 50 years from the discovery of iproniazid and imipramine, the strategy for developing novel thymoanaleptics has been based not so much on seeking more effective antidepressants with new mechanisms of action as on minimizing the unwanted side effects of monoaminergic drugs [22]. This has led to the accumulation of a mass of data on the two main problems of using monoaminergic antidepressants – the long period of time between starting treatment with thymoanaleptics to the onset of their clinical effect and the very frequent (up to 30–40%) complete absence of clinical effect [19, 21, 25, 26]. This situation provides evidence that the widely accepted monoamine theory of depression is not the whole story in the pathogenesis of depression, clarification of which is needed for more effective and fast-acting antidepressants with fundamentally new mechanisms of action. Thus, the glutamate hypothesis of depression is worthy of detailed attention; this postulates a pathogenetic role for impairments to the balance between stimulation of AMPA and NMDA receptors at the cerebral level [18, 19, 25]. The clear basis of the glutamate hypothesis is the acute antidepressant effect ("rapid-onset antidepressant-like effect") seen with the NMDA receptor antagonist ketamine, which develops after a single subanesthetic dose and is blocked by an AMPA receptor antagonist [19, 25]. The clinical use of ketamine for the treatment of depression is significantly difficult because of the widely

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known psychedelic activity of this drug [19]. Deeper study of the antidepressant activity of the Russian drug Mexidol has more potential; like ketamine, this blocks NMDA receptors [13] and has thymoanaleptic activity [5, 7] but has virtually no side effects preventing clinical application [10]. In terms of its chemical structure, Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate) is simultaneously a 3-hydroxypyridine derivative and a succinic acid derivative. The effects of these components of Mexidol on NMDA receptors currently remain to be studied. It is important to note that isolated derivatives of 3-hydroxypyridine (emoxypine; 2-ethyl-6-methyl-3-hydroxypyridine HCl) and succinic acid (Reamberin; [N-(1-deoxy-D-glucitol-1-yl)-N-methylammonium sodium succinate), like Mexidol, have marked thymoanaleptic activity [5,]7]. The antidepressant actions of emoxypine, Reamberin, and Mexidol have previously been demonstrated in rat experiments precisely following the Porsolt et al. protocol [7]. This protocol calls for three doses of test drug over the day preceding assessment of effects on behavioral despair in the forced swimming test [17, 23]. This regime of preliminary administration of drugs is used to simulate course treatment of antidepressants in clinical practice. This article provides a comparative analysis of the acute antidepressant effects of derivatives of 3-hydroxypyridine and succinic acid developing 30–45 min after single doses of test drugs. As derivatives of 3-hydroxypyridine and succinic acid are conventionally regarded as antioxidants [10–12], their acute antidepressant actions were compared with the analogous effects of α-lipoic acid (α-LA), which is a well-known corrector of oxidative stress and has thymoanaleptic activity within the framework of the Porsolt et al. protocol [6, 7].

Methods. Experiments were conducted using 150 adult mongrel rats of both genders weighing 180–240 g. Studies were carried out in compliance with international and Russian ethical standards regulating animal experiments [12]. Study drugs were given i.p. as single doses 30 min before assessment of antidepressant activity. Each drug was used at three doses extrapolated from single doses in the therapeutic range for humans and taking account of differences in relative body surface area [2, 12]. In all cases, the minimum single doses in the ranges were ½ the calculated equivalent mean therapeutic dose (EMTD). The maximum dose was 2EMTD. Emoxypine (Moscow Endocrine Factory, Russia) was used at doses of 6.25, 12.5, and 25 mg/kg. Test doses of 1.5% Reamberin solution (NTFF Polysan, Russia) were 12.5, 25, and 50 ml/kg. Mexidol (NPK Pharmasoft, Russia) doses were 12.5, 25, and 50 mg/kg. The antidepressant activity of α-lipoic acid (α-LA) was also studied; this has previously been used as a reference agent in experimental studies of the effects of derivatives of 3-hydroxypyridine and succinic acid in disorders of motivational behavior in animals with alloxan diabetes [4, 8]. α-LA (Berlitione, Berlin-Chemie/Menarini Pharma GmbH, Germany) was used at doses of 25, 50, and 100 mg/kg (½ EMTD, EMTD, and 2EMTD, respectively). The reference antidepressant was amitriptyline (Amitriptyline-AKOS; Sintez, Russia) at a dose of 2.5 mg/kg (EMTD). As the stimulation method could give false positive results on screening for potential antidepressants [23], caffeine (caffeine benzoate sodium, RUP Borisov Medicines Factory, Belarus) was used as an additional reference agent at a dose of 18 mg/kg (EMTD). All doses of study drugs were given in a final volume of 50 ml/kg (drugs were diluted as needed in 0.9% NaCl). Rats of the control group received the same volume of isotonic NaCl.

A preliminary series of experiments assessed the effects of drugs on affective status in rats immediately before modeling behavioral despair. Thus, activity in the open field test was recorded for 5 min 25 min after administration of study drugs, with monitoring of locomotion, exploratory-orientational activity, along with grooming and defecation, which are regarded as the autonomic equivalents of anxiety in rats [1, 2]. This approach yielded an integral characteristic for the affective status of rodents, whose activity in the open field test forms as a result of two equal but opposite influences – fear of the unknown space and the motivation to explore it.

Immediately after completion of testing of rats in the open field (30 min after drug administration), animals were placed in a transparent, water-filled glass cylinder (height 40 cm, cross-sectional diameter 20 cm, height of water column 13 cm, water temperature 25°C). The duration of behavioral despair was assessed in terms of the total duration of immobility in each of three 5-min intervals during a continuous 15-min session of forced swimming, and the integral duration of behavioral despair throughout the test was computed. This provided for studies of the dynamics of the onset of the acute antidepressant effect at 30–45 min after single drug doses. The algorithm was a modified Porsolt test – the original version involves 15 min of forced swimming before drug treatment, and the duration of immobility is not assessed, the procedure being used exclusively for adaptation of rats to the experimental conditions [17, 23].

Statistical analysis was performed in SPSS-17.0. Data were processed by descriptive statistics methods and are presented as median (Me) and ranges between the lower (LQ, 25th percentile) and upper (UQ, 75th percentile) quartiles. Significant between-group differences were identified using the paired Wilcoxon test and the Mann–Whitney U test (for independent sets). Statistical hypotheses were tested at the critical significance level of $p = 0.05$.

Results. Analysis of the dynamics of behavioral despair in the modified Porsolt test demonstrated a rapid increase in the duration of immobility during 15-min sessions of forced swimming (Table 1). This shows that the durations of immobility in 5-min periods II and III of the test were greater than the value in interval I by 41% and 46%, respectively (in both cases, $p = 0.005$, paired Wilcoxon test). This fact corresponds to the concept of the rapid formation of the

TABLE 1. Effects of Derivatives of 3-Hydroxypyridine and Succinic Acid on the Duration of Behavioral Despair in Rats in the Modified Porsolt Test [Me (LQ-UQ)]

Group (n = 10)	Interval in forced swimming test			Integral duration of behavioral despair, sec
	Interval I (0–5 min)	Interval II (6–10 min)	Interval III (11–15 min)	
	Duration of behavioral despair, sec			
Control	175.0 (133.3—184.8)	246.5 (223.0—260.3)	255.5 (236.0—277.0)	668.0 (606.0—714.8)
Amitriptyline (2.5 mg/kg)	**217.5* (165.8—226.8)**	246.0 (237.0—257.5)	243.0 (240.0—252.5)	716.0 (663.0—727.5)
Caffeine (18 mg/kg)	**61.0* (17.6—153.8)**	**114.0* (52.5—240.3)**	**131.0* (41.0—246.0)**	**306.0* (119.8—646.0)**
Emoxypine				
½EMTD (6.25 mg/kg)	180.0 (93.8—204.0)	236.0 (158.3—263.8)	241.0 (205.0—273.0)	635.0 (518.3—708.8)
EMTD (12.5 mg/kg)	128.5 (108.8—169.0)	207.5 (162.0—250.5)	**235.0* (195.0—250.8)**	**591.5* (488.8—642.8)**
2EMTD (25 mg/kg)	162.0 (76.5—185.5)	215.5 (148.8—251.0)	235.0 (181.5—256.5)	588.0 (446.3—681.0)
Reamberin				
½EMTD (12.5 ml/kg)	153.0 (122.5—202.5)	250.0 (145.0—268.8)	227.0 (184.3—262.5)	652.5 (451.3—681.3)
EMTD (25 ml/kg)	165.5 (138.0—195.0)	205.0 (127.3—250.3)	**213.0* (179.5—235.0)**	565.0 (483.0—676.5)
2EMTD (50 ml/kg)	133.5 (112.8—168.8)	**189.5* (165.8—207.5)**	**214.5* (190.5—251.3)**	**538.0* (476.0—604.3)**
Mexidol				
½EMTD (12.5 mg/kg)	169.0 (134.5—177.0)	**186.5* (173.0—196.5)**	239.0 (212.3—252.3)	**599.3* (531.8—613.5)**
EMTD (25 mg/kg)	**120.5* (103.8—138.8)**	220.0 (137.8—258.0)	**207.0* (172.0—231.0)**	**533.0* (496.0—575.5)**
2EMTD (50 mg/kg)	154.0 (134.3—167.3)	235.5 (176.8—261.8)	240.0 (223.5—250.0)	626.0 (565.8—652.8)
α-Lipoic acid				
½EMTD (25 mg/kg)	139.5 (130.0—166.0)	214.0 (186.5—245.0)	**236.0* (192.0—240.3)**	**607.0* (513.0—633.5)**
EMTD (50 mg/kg)	161.0 (154.0—168.8)	**201.0* (179.8—209.5)**	244.0 (227.5—270.8)	**607.5* (567.5—631.5)**
2EMTD (100 mg/kg)	177.5 (157.5—186.3)	201.5 (195.0—234.8)	242.0 (227.8—257.3)	629.0 (608.0—660.0)

In place of study drugs, control rats received the same volume of 0.9% NaCl solution; *$p \le 0.05$ compared with controls (Mann–Whitney U test).

skill of immobility as an optimum resource-sparing adaptation strategy for the unavoidable stress of forced swimming [23]. This version of adaptation is due to elevated stimulation of glucocorticoid receptors in the limbic structures of the brain on the background of stress-induced activation of the hypothalamo-hypophyseal-adrenocortical axis [20]. It is important to emphasize that depression in humans is also regarded as a sign of adaptation to difficult problems and is linked with the development of stress [16]. This view allows immobility in the forced swimming test to be regarded as a reaction homologous to depression in humans. Porsolt et al. believe that behavioral despair in forced swimming in rodents is homologous to hypothymia [17], which is one of the main symptoms of depression in humans [15].

Behavioral despair in the modified Porsolt test demonstrated high resistance to the thymoanaleptic action of amitriptyline (Table 1). This was apparent as the absence of an antidepressant effect with amitriptyline at the EMTD dose, use of which in the framework of the original Porsolt protocol in previous studies induced an almost twofold decrease in immobility [7]. Furthermore, administration of amitriptyline at the EMTD dose produced a paradoxical increase, by 24%, in the duration of immobility in the first 5-min period of forced swimming (Table 1). This phenomenon is probably linked with the sedative action of amitriptyline, apparent as decreased motor and exploratory-orientational activity in rats in the open field, by 23–40% (Table 2). These data do not exclude the use of this modification of the Porsolt test for screening classical (monoaminergic) antidepressants at significantly elevated doses. This modified approach demonstrated its validity for assessment of the antidepressant activity of amitriptyline, the dose of which

TABLE 2. Effects of Derivatives of 3-Hydroxypyridine and Succinic Acid on the Behavior of Rats in the Open Field [Me (LQ-UQ)

Group (n = 10)	Movement activity (square crossings)	Orientational activity (vertical rearings)	Exploratory activity (glances through apertures)	Grooming (number of selfcare acts)	Defecations (number of fecal boluses)
Control	41.5 (38.0—45.8)	7.5 (5.8—10.0)	9.5 (5.0—11.0)	2.0 (1.0—3.0)	3.0 (0.8—3.3)
Amitriptyline (2.5 mg/kg)	**32.0* (26.5—42.5)**	**4.5* (2.8—7.3)**	**6.0* (3.8—8.0)**	**0* (0—1.0)**	**0* (0—0)**
Caffeine (18 mg/kg)	**60.0* (55.5—65.3)**	**13.0* (7.8—14.3)**	**15.0* (10.5—16.0)**	**0* (0—1.0)**	**0* (0—0)**
Emoxypine					
½EMTD (6.25 mg/kg)	**54.0* (42.3—58.5)**	8.0 (4.5—14.0)	7.0 (5.8—12.0)	**0* (0—1.3)**	**0* (0—0)**
EMTD (12.5 mg/kg)	46.5 (38.3—52.5)	8.0 (5.8—9.0)	7.0 (5.0—8.3)	**0.5* (0—1.0)**	**1.0* (0—1.0)**
2EMTD (25 mg/kg)	48.0 (38.8—51.8)	9.5 (7.0—10.5)	8.5 (6.5—9.8)	**1.0* (0—1.0)**	**0.5* (0—1.0)**
Reamberin					
½EMTD (12.5 ml/kg)	32.0 (28.0—43.3)	**5.0* (4.8—7.3)**	8.0 (5.8—11.0)	1.0 (1.0—2.3)	**1.0* (0—2.0)**
EMTD (25 ml/kg)	**33.5* (28.8—38.0)**	**4.5* (3.5—7.0)**	7.5 (4.8—10.0)	**0.5* (0—2.0)**	**0* (0—0.3)**
2EMTD (50 ml/kg)	38.5 (33.3—45.3)	**4.0* (1.8—8.3)**	8.0 (6.8—9.3)	1.0 (0—2.3)	**1.0* (0—1.0)**
Mexidol					
½EMTD (12.5 mg/kg)	40.5 (32.0—45.5)	6.0 (3.8—11.0)	10.0 (3.7—12.5)	1.0 (1.0—2.0)	**1.0* (0—1.0)**
EMTD (25 mg/kg)	39.0 (25.8—41.3)	4.5 (3.5—10.0)	**5.0* (3.8—7.0)**	**1.0* (0—1.0)**	1.0 (0.8—2.0)
2EMTD (50 mg/kg)	39.5 (27.5—44.8)	**3.5* (2.8—8.3)**	7.5 (4.0—10.3)	**1.0* (0—1.3)**	**1.0* (0—1.0)**
α-Lipoic acid					
½EMTD (25 mg/kg)	**28.5* (23.8—29.0)**	6.5 (3.8—7.0)	8.0 (5.5—9.3)	**1.0* (0—1.3)**	**0.5* (0—1.0)**
EMTD (50 mg/kg)	**25.0* (23.3—27.5)**	**4.5* (3.8—5.5)**	**6.0* (4.0—7.0)**	**1.0* (0—1.0)**	**1.0* (0—1.3)**
2EMTD (100 mg/kg)	**12.0* (10.0—20.0)**	**2.0* (0.8—3.0)**	**2.5* (1.0—3.0)**	**0* (0—0.3)**	1.5 (0—2.3)

This table shows results from recording behavioral acts in the 5-min observation period in the open field; in place of study drugs, control rats received the same volume of 0.9% NaCl solution; *$p \leq 0.05$ compared with controls (Mann–Whitney U test).

(10 mg/kg) was four times greater than the EMTD [14]. The significant increase in the amitriptyline dose evidently eliminates the risk of false negative results in assessments of antidepressant activity. The false negative result using amitriptyline at the lower dose (2.5 mg, EMTD) is probably due to predominance of the concomitant (sedative) action of this drug over its main (antidepressant) action.

Single doses of caffeine at the EMTD, in contrast to the same dose of amitriptyline, led to reductions in all measures of immobility in the process of forced swimming, by 49–65% (Table 1), on the background of increased locomotion and exploratory-orientational activity in the open field by 45–73% (Table 2). These observations provide evidence of the high sensitivity of the modified Porsolt method to substances with stimulatory actions (caffeine) and the complete absence of a thymoanaleptic response to the EMTD of an antidepressant with the classical (monoaminergic) mechanism of action (amitriptyline). This circumstance illustrates the high risk of false positive and false negative results on screening potential antidepressants because of their concomitant stimulatory or sedative actions, respectively. To minimize this risk of false results, data from the modified Porsolt test must be compared with measures of locomotion and exploratory-orientational activity in the open field test. Parameters such as grooming and defecation, which are indirect indicators of anxiety in rodents [1], cannot differentiate psychostimulators from thymoanaleptics with their concomitant sedative effects. This is evidenced by the fact that changes in grooming and defecation are altered in the same direction by caffeine and amitriptyline, which com-

pletely suppress these forms of activity in the open field test (Table 2). These facts evidently reflect the inverted U-shaped relationship between grooming and defecation and the level of arousal, caffeine-induced increases in which, like amitriptyline-induced decreases, lead to different decreases in these types of behavior in the open field. We add that suppression of defecation by amitriptyline at the EMTD may to some extent be associated with its m-cholinolytic side effect.

Overall, the experimental results obtained with reference agents (amitriptyline and caffeine) demonstrated low sensitivity and low specificity of the modified Porsolt test in relation to antidepressants with the classical (monoaminergic) mechanism of action. This allowed the proposed modified forced swimming test to be seen as the preferred tool for screening fast-acting atypical antidepressants. Exclusion of false positive and false negative results from screening requires prior evaluation of the stimulatory or sedative activity of potential thymoanaleptics in the open field test. It is important to note that testing of animals in the open field is a weakly stress-inducing situation, which can be reflected in the duration of episodes of immobility of rats during forced swimming. It is entirely likely that being in the open field prior to application of the modified Porsolt test plays the same role as the prior session of forced swimming in the framework of the classical version of this test. Prior 15-min swimming, producing prolonged immobility in the classical Porsolt test [17, 23] is evidently a stronger stressor than 5 min in the open field.

Single doses of all the derivatives of 3-hydroxypyridine and succinic acid studied here significantly reduced immobility in the modified Porsolt test (Table 1) and had significant effects on the activity of the animals in the open field (Table 2). A relatively later onset of the acute antidepressant effect was noted for emoxypine, which decreased the duration of behavioral despair in period III of forced swimming by 8% and decreased the integral measure of immobility by 11%. This effect was seen only at the intermediate dose (EMTD), which had no effect on the movement or exploratory-orientational activity of the rats in the open field. Single doses of emoxypine at the minimum dose (½EMTD) had no effect on immobility during forced swimming (Table 1) but induced a clear stimulatory effect in the open field (Table 2). Emoxypine used at the minimal dose increased movement activity in the rats by 30%, which is entirely comparable with the 27% increase in locomotion of mice after single doses of emoxypine at the ½EMTD dose [5]. These data provide evidence that reductions in immobility in response to the derivative of 3-hydroxypyridine alone (emoxypine) was independent of its stimulatory activity and can be regarded as a manifestation of the acute antidepressant effect. Similar results have been obtained previously in experiments on mice [5].

Single doses of the derivative of succinic acid alone (Reamberin) induced an acute antidepressant effect which increased with increases in dose and reached statistical significance using relatively high doses (EMTD and 2EMTD). Use of Reamberin at the EMTD, like the corresponding emoxypine dose, produced a significant decrease in the duration of immobility in period III of forced swimming. This measure of behavioral despair decreased by 17% in response to Reamberin, which, in contrast to the corresponding effect of emoxypine, did not produce any significant changes in the integral indicator of immobility. Administration of Reamberin at the maximum dose (2EMTD) induced an accelerated and more marked antidepressant effect. This was apparent as a reduction in behavioral despair in periods II and III of forced swimming, by 23% and 16%, respectively, along with a reduction in the integral indicator of immobility, by 19%. It is important to emphasize that Reamberin, in contrast to caffeine, decreased behavioral despair (Table 1) on the background of a concomitant sedative effect, which was apparent over the whole range of doses used (Table 2). All doses of Reamberin suppressed orientational activity of rats in the open field, by 33–47%. Use of Reamberin at the intermediate dose (EMTD) additionally decreased the animals' movement activity, by 19%. Data on the sedative influence of Reamberin corresponded to results from previous studies in mice [5] and allow the reduction in behavioral despair in response to this drug to be regarded as an intrinsic manifestation of its antidepressant activity.

An analogous combination of effects was seen with Mexidol, which is a derivative of both 3-hydroxypyridine and succinic acid (Tables 1 and 2). Single doses of this drug at the minimum dose (½EMTD) led to a 24% decrease in immobility in period II of forced swimming and decreased the integral indicator of behavioral despair by 10%. Significant acceleration and increases in the extent of this effect were seen using the intermediate dose (EMTD) of Mexidol. This was apparent as a reduction in immobility by 31% in period I of forced swimming, along with a 19% decrease in behavioral despair in period III of the test and a 20% decrease in the integral indicator of immobility. The increase in the Mexidol dose to 2EMTD led to loss of its influence on immobility in the modified Porsolt test (Table 1). Studies of the effects of Mexidol on behavior in rats in the open field demonstrated the sedative influence of this drug at the intermediate and maximum doses. As shown (Table 2), administration of Mexidol at the EMTD decreased exploratory activity in animals by 47%, while use at 2EMTD decreased orientational activity in rats by 53%. The overall dataset allows Mexidol to be regarded as a fast-acting antidepressant with a concomitant sedative action.

An identical profile of psychotropic activity was demonstrated for α-LA, which reduced behavioral despair in forced swimming and reduced the activity of the animals in the open field (Tables 1 and 2). The thymoanaleptic action of α-LA, like that of Mexidol, was seen in the relatively low dose range (½EMTD and EMTD). Single doses of α-LA at the minimum dose decreased the duration of immobility in period III of forced swimming by 8% and decreased the

overall indicator of behavioral despair by 9%. Use of α-LA at the intermediate dose produced an 18% reduction in the immobility indicator in period II of the modified Porsolt test and decreased the integral indicator of immobility to the level reached on administration of the minimum dose of this drug (Table 1). The sedative action of α-LA was apparent as suppression of the main forms of behavior of rats in the open field in a manner directly dependent on the dose used (Table 2). This applies to the animals' movement activity, which decreased significantly, by 31–71%, over the whole range of study doses, and to the rats' exploratory-orientational activity, which decreased significantly, by 37–74%, on use of relatively high doses (2EMTD and EMTD). It is entirely possible that the marked sedative action of α-LA may limit the manifestation of its antidepressant activity in the modified forced swimming test. The correctness of this suggestion is illustrated by the fact that at the maximum dose (2EMTD), α-LA produced the most marked sedative effect and lost its influence on behavioral despair (Tables 1 and 2).

Comparative analysis of the dynamics of the acute antidepressant action of study drugs illustrates the clear advantage of succinate-containing substances (Reamberin and Mexidol) over the derivative of 3-hydroxypyridine alone (emoxypine). This was apparent as the higher rates of onset of the thymoanaleptic effect of succinic acid derivatives. Table 1 shows that reductions in behavioral despair due to Reamberin and Mexidol developed significantly earlier than on use of emoxypine.

The features of the actions of study drugs on the behavior of rats in the open field requires separate consideration. In particular, this applies to changes in indirect measures of anxiety – grooming and defecation. Tables 1 and 2 show that emoxypine, Reamberin, Mexidol, and α-LA, like amitriptyline and caffeine, significantly decreased these parameters independently of their influences on the main measures of behavior in the open field and the duration of behavioral despair. This is evidence that recording grooming and defecation has little value in assessing the effects of potential antidepressants on behavior in rats. Grounded assessments of the stimulatory or sedative actions of likely thymoanaleptics given as single doses require consideration only of their influences on the main indicators of behavior in the open field (movement and exploratory-orientational activity).

It should be emphasized that repeated administration of study drugs produces tolerance to their sedative effects in the framework of the classical Porsolt protocol, not infrequently with inversion of effects on movement and exploratory-orientational activity. This applies to amitriptyline, which lost its suppressing action on the main measures of behavior in the open field after three doses, and to succinate-containing drugs (Reamberin and Mexidol), which lost their sedative actions and acquired stimulatory activity in this same administration regimen [7]. An analogous inversion of the sedative effect was demonstrated for α-LA given three times at the minimum dose [7]. Qualitative indicators of the direction of the effects of these drugs on behavior in the open field are due to significant increases in thymoanaleptic effects during treatment courses with these drugs. In contrast to single doses of study drugs, (Table 1), courses of drugs decreased behavioral despair over the whole range of doses used [7]. This allows activation of rats in the open field to be regarded as resulting from an increase in motivation to explore the unfamiliar space because of escalation of the thymoanaleptic effects of study drugs [7]. It is important to add that the onset of this effect started less than an hour after single doses of derivatives of 3-hydroxypyridine and succinic acid, and the presence of succinate in the structure was accompanied by sedative actions limiting the manifestation of antidepressant activity. This situation excludes the risk of false positive results and illustrates the high specificity of the acute antidepressant effects (rapid-onset antidepressant-like effect) of succinate-containing drugs (Reamberin and Mexidol).

A different situation was seen on analysis of the effects of emoxypine. This is a drug which is a derivative of 3-hydroxypyridine alone; it increased movement activity in rats in the open field after both single doses (Table 2) and when given as courses [7]. It should be emphasized that single doses of emoxypine increased locomotion and decreased behavioral despair only at particular doses (the ½EMTD and EMTD, respectively) (Tables 1 and 2), while courses induced these same effects over the whole range of doses studied [7]. The parallel increase in the doses producing the stimulatory and thymoanaleptic actions of emoxypine when given as courses means that the risk of false positive results cannot be excluded completely on assessment of antidepressant activity. This applies particularly to the use of emoxypine at relatively low doses (½EMTD and EMTD), which have the most marked stimulatory actions in both rats [7] and mice [5]. In addition, a previous study in mice showed that single doses of emoxypine at the maximum dose (2EMTD) induced a true antidepressant effect on the background of a concomitant sedative action [5]. This fact, along with the mismatch of the doses at which emoxypine had stimulatory and antidepressant actions when given as single doses (Tables 1 and 2), makes it impossible to exclude the possibility that this drug has a thymoanaleptic effect. It is entirely possible that emoxypine, like imipramine, should be positioned as an antidepressant with concomitant stimulatory activity. It may be that the concomitant stimulatory action of emoxypine gives it the advantage over Mexidol in terms of efficacy in correcting affective disorders in patients with diabetes mellitus [3].

The multiplicity of the likely mechanisms for the thymoanaleptic effects of derivatives of 3-hydroxypyridine and succinic acid requires separate assessment. 2,6-Dialkyl-3-hydroxypyridine derivatives are known to be able to stimulate serotonin release and suppress its reuptake by synaptosomes in the rat brain [11]. This is evidence that there may be a common mechanism for the thymoanaleptic action of derivatives of 3-hydroxypyridine (emoxypine and Mexidol)

and classical antidepressants of the selective serotonin reuptake inhibitor (SSRI) group. In addition, the antidepressant actions of study drugs depend directly on their insulin-potentiating activity [6], which fits into the framework of current concepts of the role of insulin resistance in the pathogenesis of depression and the thymoanaleptic potential of insulin sensitizers [24]. This pattern was clearly apparent for succinate-containing drugs (Reamberin and Mexidol), which induce the most marked insulin-potentiating and thymoanaleptic effects [6]. It should be added that all derivatives of 3-hydroxypyridine and succinic acid studied here have antiglucocorticoid activity [9], which is regarded as one of the mechanisms of the antidepressant action [27]. The antagonism of Mexidol for NMDA receptors [13] requires no less attention; extreme stimulation of these receptors is an important mechanism in development of depression [19]. An important perspective in studies of the mechanisms of the rapid antidepressant effects of emoxypine and Reamberin is linked with the as yet incompletely evaluated influences on NMDA receptors.

Overall, the results of this study, along with published data, allows the Russian derivatives of 3-hydroxypyridine and succinic acid to be regarded as potential antidepressants with multiple mechanisms of action and rapid onset of thymoanaleptic effects. The rate of onset of the acute antidepressant effect and the presence of an additional stimulatory or sedative effect depend on the chemical structure of the derivatives of 3-hydroxypyridine and succinic acid. The isolated 3-hydroxypyridine derivative emoxypine was less active than the succinic acid derivatives (Reamberin and Mexidol) in terms of the rate of onset of the antidepressant effect and had a stimulatory action after single subantidepressant doses. The acute antidepressant actions of Reamberin and Mexidol develop on the background of concomitant sedative effects.

REFERENCES

1. Ya. Buresh, O. Bureshova, and J. P. Houston, *Methods and Basic Experiments in Research on the Brain and Behavior*, Vysshaya Shkola, Moscow (1991).
2. I. A. Volchegorskii, I. I. Dolgushin, O. L. Kolesnikov, and V. E. Tseilikman, *Experimental Modeling and Laboratory Evaluation of the Body's Adaptive Reactions*, Chelyabinsk State Pedagogical University Pres, Chelyabinsk (2000).
3. I. A. Volchegorskii and N. V. Mester, "Effects of antioxidants of the 3-hydroxypyridine group on depression in patients with diabetes mellitus," *Klin. Med.*, **85**, No. 2, 40–45 (2007).
4. I. A. Volchegorskii, L. M. Rassokhina, and I. Yu. Miroshnichenko, "Antidepressant actions of α-lipoic acid and Reamberin in alloxan diabetes in mice," *Prob. Endokrinol.*, **55**, No. 4, 20–24 (2009).
5. I. A. Volchegorskii, I. Yu. Miroshnichenko, L. M. Rassokhina, and R. M. Faizullin, "Studies of the antidepressant activity of derivatives of 3-hydroxypyridine and succinic acid in experiments on mice," *Eksperim. Klin. Farmakol.*, **76**, No. 7, 6–10 (2013).
6. I. A. Volchegorskii, I. Yu. Miroshnichenko, L. M. Rassokhina, and R. M. Faizullin, "The insulin-potentiating and antidepressant actions of derivatives of 3-hydroxypyridine and succinic acid," *Eksperim. Klin. Farmakol.*, **77**, No. 5, 6–9 (2014).
7. I. A. Volchegorskii, I. Yu. Miroshnichenko, L. M. Rassokhina, et al., "Experimental studies of the antidepressant action of derivatives of 3-hydroxypyridine and succinic acid," *Zh. Nevrol. Psikhiat.*, **2**, 48–52 (2015).
8. I. A. Volchegorskii, I. Yu. Miroshnichenko, L. M. Rassokhina, et al., "Anxiolytic and antidepressant actions of derivatives of 3-hydroxypyridine and succinic acid in alloxan diabetes," *Ros. Fiziol. Zh.*, **101**, No. 3, 258–267 (2015).
9. I. A. Volchegorskii, I. Yu. Miroshnichenko, L. M. Rassokhina, et al., "Antianhedonistic actions of derivatives of 3-hydroxypyridine and succinic acid," *Ros. Fiziol. Zh.*, **102**, No. 11, 1312–1322 (2016).
10. T. A. Voronina, "Mexidol: spectrum of pharmacological effects," *Zh. Nevrol. Psikhiat.*, **12**, 86–90 (2012).
11. E. S. Kovaleva, L. L. Prilipko, K. O. Muranov, and V. E. Kagan, "Actions of antioxidants on ^{3}H-serotonin release from rat brain synaptosomes," *Byull. Eksperim. Biol. Med.*, **96**, No. 10, 55–57 (1983).
12. A. N. Mironov, *Handbook for Preclinical Studies of Drugs*, Grif i K, Moscow (2012).
13. V. G. Motin, V. V. Yasnetsov, A. A. Zabozlaev, et al., "An electrophysiological study of the mechanism of action of Mexidol," *Eksperim. Klin. Farmakol.*, **75**, No. 1, 3–7 (2012).
14. S. B. Seredenin, G. M. Molodavkin, M. V. Voronin, and T. A. Voronina, "Antidepressant action of afobazole in the Porsolt and Nomura tests," *Eksperim. Klin. Farmakol.*, **72**, No. 1, 19–21 (2009).
15. A. B. Smulevich, *Depression in Somatic and Mental Diseases*, Medical Information Agency, Moscow (2003).
16. P. W. Andrews and J. A. Thomson, "The bright side of being blue: depression as an adaptation for analyzing complex problems," *Psychol. Rev.*, **116**, No. 3, 620–654 (2009).
17. J. J. Buccafusco, *Methods of Behavioral Analysis in Neuroscience*, CRC Press LLC, London, New York (2009).
18. Shi Yu Chan, E. Matthews, and P. W. J. Burne, "ON or OFF?: Modulating the N-methyl-D-aspartate receptor in major depression," *Front .Mol. Neuroscience*, **9**, 169 (2016).
19. K. E. DeWilde, C. F. Levitch, J. W. Murrough, et al., "The promise of ketamine for treatment-resistant depression: current evidence and future directions," *Ann. N. Y. Acad. Sci.*, **1345**, 47–58 (2015).
20. E. R. Kloet and M. L. Molendijk, "Coping with the forced swim stressor: Towards understanding an adaptive mechanism," *Neural Plast.*, **2016**, 6503162 (2016).
21. M. R. Levinstein and B. A. Samuels, "Mechanisms underlying the antidepressant response and treatment resistance," *Front. Behav. Neurosci.*, **8**, 208 (2014).
22. F. Lopez-Munoz and C. Alamo, "Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today," *Curr. Pharm. Des.*, **15**, No. 14, 1563–1586 (2009).
23. R. D. Porsolt, G. Anton, N. Blavet, and M. Jalfre, "Behavioural despair in rats: a new model sensitive to antidepressant treatments," *Eur. J. Pharmacol.*, **47**, No. 4, 379–391 (1978).
24. N. L. Rasgon, H. A. Kenna, K. E. Williams, et al., "Rosiglitazone add-on in treatment of depressed patients with insulin resistance: a pilot study," *Sci. World J.*, **10**, 321–328 (2010).
25. I. J. Shin, S. U. Son, H. Park, et al., "Preclinical evidence of rapid-onset antidepressant-like effect in *Radix polygalae* extract," *PLoS One*, **9**, No. 2, e88617 (2014).
26. D. A. Slattery and J. F. Cryan, "Using the rat forced swim test to assess antidepressant-like activity in rodents," *Nat. Protoc.*, **7**, No. 6, 1009–1014 (2012).
27. A. C. Wulsin, J. P. Herman, and M. B. Solomon, "Mifepristone decreases depression-like behavior and modulates neuroendocrine and central hypothalamic-pituitary-adrenocortical axis responsiveness to stress," *Psychoneuroendocrinology*, **35**, No. 7, 1100–1112 (2010).