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The Antidepressant Actions of 3-Hydroxypyridine and Succinic Acid Derivatives in Experimental Studies

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Objective. To study the antidepressant activity of original Russian derivatives of 3-hydroxypyridine and succinic acid (emoxypine, Reamberin, and Mexidol) in experiments on rats. **Materials and methods.** The effects of emoxypine, Reamberin, and Mexidol on the duration of "despair behavior" were studied in rats in the Porsolt forced swimming test. The effects of study drugs on the animals' behavior in the open field test were also studied. Amitriptyline and α-lipoic acid were used as reference agents. **Results and conclusions.** Three optimum doses of each of the study drugs, corresponding to the therapeutic range in humans, were found to decrease the duration of despair behavior in the Porsolt test. This effect of emoxypine, Reamberin, Mexidol, and α-lipoic acid provide evidence of their antidepressant activity, the extent of which depended on the actions of the study drugs in the open field. Reamberin and α-lipoic acid, the maximal doses of which either had no effect on orientational activity in the open field (Reamberin) or suppressed it (α-lipoic acid), were no less active than amitriptyline in terms of the extent of the antidepressant effect. 3-Hydroxypyridine derivatives (emoxypine and Mexidol), which had stimulatory actions on activity in the open field test, were significantly less active than amitriptyline in decreasing the duration of despair behavior.

Keywords: 3-Hydroxypyridine and succinic acid derivatives, antidepressant actions.

Russian derivatives of 3-hydroxypyridine and succinic acid (emoxypine, Reamberin, and Mexidol) have significant therapeutic potential, such that they should be included in complex treatment schemes for socially significant and very common human diseases. This is evidenced by the efficacy of 3-hydroxypyridine and succinic acid derivatives in cerebrovascular and cardiovascular pathologies, diabetic and compression neuropathies, withdrawal states, pulmonary tuberculosis, and primary open-angle glaucoma [1–6]. The wide spectrum of the therapeutic effects of emoxypine, Reamberin, and Mexidol are to a significant extent related to their antioxidant activities [7–9]. Use of these drugs often leads not only to correction of the signs of the main disease, but also decreases in the symptoms of comorbid depression [10, 11]. The suggestion that 3-hydroxypyridine and succinic acid derivatives, which are positioned as antioxidants [7, 9], have antidepressant activity, is consistent with data reported on [12] on the ability of antioxidants (particularly 2-methyl-6-ethyl-3-hydroxypyridine) to increase serotonin release and suppress its reuptake by synaptosomes in the rat brain. It remains possible that this action of 3-hydroxypyridine and succinic acid derivatives may correlate with the well-known neurochemical component of the pathogenesis of depression – the functional deficit of serotoninergic neurotransmission at the cerebral level. This mechanism underlies the antidepressant actions of selective serotonin reuptake inhibitors (SSRI). The clinical efficacy of one SSRI (fluvoxamine) has been shown to increase significantly on the background of treatment with Mexidol [13], which is a derivative of both 3-hydroxypyridine and succinic acid [8]. This can be regarded as an additional argument suggesting that emoxypine, Reamberin, and Mexidol have antidepressant activity.

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The aim of the present work was to study the antidepressant activity of 3-hydroxypyridine and succinic acid derivatives – emoxypine, Reamberin, and Mexidol – in experiments on rats.

Materials and Methods

Experiments were performed on 314 adult mongrel rats of both genders, weighing 140–180 g.

Studies were conducted in compliance with international ethical norms regulating experiments on animals [14]. In accordance with recommendations [15], drugs were given i.p. three times – 24 h, 4 h, and 30 min before assessment of their antidepressant activities. Each drug was used at three doses, equivalent to individual doses in the human therapeutic range, allowing for differences in in relative body surface area [16]. In all cases, the minimum single doses in the study range was half the calculated equivalent mean therapeutic dose (EMTD). The maximum dose was double the EMTD.

Emoxypine (2-ethyl-6-methyl-3-hydroxypyridine hydrochloride (Moscow Endocrine Factory, Russia) was used at doses of 6.25, 12.5, and 25 mg/kg. The study doses of 1.5% Reamberin (N-(1-deoxy-D-glucitol-1-yl)-N-methylammonium sodium succinate, Polisan, St. Petersburg) were 12.5, 25, and 50 ml/kg. Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate (Farmasoft, Russia) was used at doses of 12.5, 25, and 50 mg/kg.

The antidepressant activity of α-lipoid acid (α-LA) were also studied; this compound has previously been used as a reference agent for experimental studies of the effects of 3-hydroxypyridine and succinic acid derivatives on disorders of motivated behavior in alloxan diabetes [17, 18]. α-LA (Berlition, Berlin Chemie/Menarini Pharma, Germany) was used at doses of 25, 50, and 100 mg/kg (½EMTD, EMTD, and 2EMTD, respectively).

The reference antidepressant was amitriptyline (amitriptyline-AKOS, Sintez, Kurgan) at a dose of 2.5 mg/kg (EMTD).

All doses of study drugs were given in a final volume of 50 ml/kg (where required, drugs were diluted with 0.9% NaCl).

Rats of the control group received the same volume of isotonic NaCl solution. In accordance with recommendations [15], the study design was based on integrated evaluation of repeat "sub-experiments," each of which used 14–28 rats (1–2 from each group). This approach ensures uniform experimental conditions for all study groups and allows a single control group to be used for 1–15 experimental groups [19].

The antidepressant actions of drugs were assessed in terms of their effects on the duration of despair behavior in the Porsolt test [19]. Rats were subjected to forced swimming in transparent glass cylinders filled with water (40 cm high, cross-sectional diameter 20 cm, water column height 13 cm, water temperature 25°C). Rats were adapted to the experimental conditions using preliminary 15-min sessions of forced swimming one day before assessment of the antidepressant actions of drugs. This session was used exclusively to acclimatize the animals to the situation and not for recording of despair behavior. After the preliminary forced swimming session, the animals were removed from the water and dried using a soft towel. This was followed immediately by the first drug injection, and rats were then placed in standard conditions with free access to water and food. The next day, after two repeat doses of drug (4 h and 30 min before assessment of antidepressant activity), the animals were again placed in the water-filled glass cylinders. The total duration of the second session of forced swimming was 5 min, during which the total duration of periods of immobility was measured (despair behavior) [19].

A separate (additional) series of experiments was performed to assess the behavior of rats in the open field 25 min after the last (third) drug injection. The behavior of the rats in the open field was recorded for 5 min in terms of locomotion, exploratory-orientational activity, grooming, and defecation, which is regarded as the autonomic equivalent of anxiety in rats [16]. This approach yields an integral characteristic of affective status in rodents, whose activity in the open field is formed by the equal actions of two opposite tendencies – the fear of the unfamiliar space and the motivation to explore it. The time period between the last injection and placing the animals in the actograph (25 min) and the test duration (5 min) allowed changes in the animals' emotional state at the start of forced swimming to be assessed, as modeled in the main (separate) series of experiments.

Statistical analysis was performed using SPSS-13.0. Data were processed by descriptive statistics methods and presented as arithmetic means and their standard errors ($M \pm m$). Significant intergroup differences were identified using the Mann–Whitney U test. Interactions were studied by calculating Spearman correlation coefficients (r_s). Statistical hypotheses were tested at a critical significance level of $p = 0.05$.

Results and Discussion

All study drugs significantly decreased the duration of immobility in the Porsolt test (see Table 1). With Mexidol and α-LA, this effect was characterized by a U-shaped dose-response relationship. In the case of Reamberin, the reduction in despair behavior increased with increasing dosage. Emoxypine, conversely, produced the largest reduction in immobility using the smallest dose, with a weak tendency to a decrease in the effect at the largest dose. It is important to emphasize that the nonlinear nature of the dose-effect relationship and the maximum action using relatively low doses are typical of thymoanaleptics [15]. The largest dose of Reamberin and all doses of α-LA had antidepressant activity, equal to that of amitriptyline 2.5 mg/kg ($p = 0.093$–0.621). In other cases, study drugs were less active than amitriptyline in terms of the ability to decrease the duration of despair behavior $p < 0.001$–0.010).

Assessment of the behavior of rats in the open field immediately before modeling of despair behavior demon-

TABLE 1. Effects of 3-Hydroxypyridine and Succinic Acid Derivatives on Behavior in an Open Field and the Duration of Immobility in Rats in the Despair Behavior Test Using the Porsolt Method ($M \pm m$)

Group	Open field						n	Duration of despair behavior, sec
	n	horizontal activity (squares crossed)	orientational activity (vertical rearings)	exploratory activity (glances through apertures)	grooming	defecation		
Control	13	43.15 ± 3.50	8.46 ± 0.97	10.92 ± 0.71	1.38 ± 0.33	0.54 ± 0.31	12	254.67 ± 9.99
Amitriptyline (2.5 mg/kg)	13	37.08 ± 5.16	7.46 ± 0.93	7.85 ± 1.20	1.31 ± 0.26	0*	12	132.42 ± 9.64*
Emoxypine								
½EMTD (6.25 mg/kg)	12	65.67 ± 4.17*	12.50 ± 1.28*	18.00 ± 1.15*	0.33 ± 0.19*	0*	10	191.10 ± 13.6*
EMTD (12.5 mg/kg)	12	67.08 ± 4.66*	11.33 ± 1.38	16.00 ± 1.28*	1.08 ± 0.34	0.42 ± 0.34	10	192.30 ± 14.1*
2EMTD (25 mg/kg)	12	60.42 ± 3.85*	11.83 ± 1.02	13.75 ± 1.16	1.58 ± 0.45	0.75 ± 0.25	10	200.80 ± 6.92*
Reamberin								
½EMTD (12.5 ml/kg)	12	68.17 ± 5.17*	14.83 ± 1.75*	16.25 ± 1.54*	0.42 ± 0.23*	1.00 ± 0.35	10	196.40 ± 13.5*
EMTD (25 ml/kg)	12	63.17 ± 3.66*	11.58 ± 1.11*	15.25 ± 0.85*	1.75 ± 0.55	0.50 ± 0.26	10	172.20 ± 15.5*
2EMTD (50 ml/kg)	12	50.83 ± 5.82	11.00 ± 1.71	12.83 ± 1.72	1.25 ± 0.37	0*	10	162.90 ± 12.4*
Mexidol								
½EMTD (12.5 mg/kg)	12	61.50 ± 4.54*	10.67 ± 1.28	15.75 ± 1.14*	0.92 ± 0.40	0.75 ± 0.25	10	186.00 ± 8.41*
EMTD (25 mg/kg)	12	57.25 ± 2.87*	11.25 ± 1.34	16.08 ± 1.67*	1.25 ± 0.35	0.50 ± 0.26	10	177.90 ± 9.30*
2EMTD (50 mg/kg)	12	52.83 ± 4.27	9.25 ± 1.74	15.42 ± 1.63*	2.08 ± 0.56	0.92 ± 0.31	10	196.30 ± 9.58*
α-Lipoic acid								
½EMTD (25 mg/kg)	12	61.67 ± 4.40*	10.83 ± 0.98	16.00 ± 1.80*	1.83 ± 0.34	0.25 ± 0.13	10	141.00 ± 12.7*
EMTD (50 mg/kg)	12	48.58 ± 3.60	8.75 ± 1.16	13.92 ± 2.43	1.00 ± 0.28	0.50 ± 0.19	10	120.50 ± 13.65*
2EMTD (100 mg/kg)	12	14.67 ± 4.07*	3.33 ± 1.23*	4.17 ± 1.07*	0.58 ± 0.29*	0.08 ± 0.08	10	134.40 ± 13.5*

Notes. In place of amitriptyline and study drugs, control rats received the same volume of 0.9% NaCl solution; *significant differences compared with control group ($p < 0.05$; Mann–Whitney U test).

strated an increase in the animals' activity in response to the study 3-hydroxypyridine and succinic acid derivatives (see Table 1). This applied primarily to emoxypine, three doses of which significantly increase the duration of activity of rats at all doses. Use of the lowest dose of emoxypine induced additional increases in all measures of exploratory-orientational activity with significant increases in measures of anxiety reactions (grooming and defecation). further increases in dose led to gradual reductions in in the stimulatory effects of emoxypine. This was apparent as the absence of any significant increase in exploratory activity in the rats using the intermediate dose of emoxypine and complete loss of its effect on exploratory-orientational activity at the highest dose. Use of emoxypine at relatively high doses (EMTD, 2EMTD) had no effect on the intensity of grooming or anxiogenic defecation in rats. These data provided evidence that the effects of emoxypine on behavior in the open field and the duration of despair behavior in the Porsolt test had the same type of dose relationship, with the maximum effect using the smallest dose. It is possible that the thymoanaleptic action of emoxypine supports the predominance of the motivation to explore the unfamiliar space over the concomitant anxiety, thus promoting activation of the animals in the open field. It is no less likely that changes in the balance between the exploratory motivation and fear of the unfamiliar space may to some extent be linked with the possible tranquilizing action of emoxypine.

Three doses of Reamberin at relatively low doses (½EMTD, EMTD) also induced increases in the movement and exploratory-orientational activity of the animals in the open field. Administration of Reamberin at the lowest dose, as with use of emoxypine at ½EMTD, led to an additional increase in grooming intensity. The maximum Reamberin dose differed from emoxypine at 2EMTD by completely preventing anxiogenic defecation and had no effect on the other measures of the rats' behavior in the open field. α-LA produced a more marked pattern in the same direction. As shown in Table 1, the smallest dose of this agent had a marked stimulatory action on movement and exploratory activity in rats, while the largest dose decreased measures of the animals' behavior on the background of a significant reduction in grooming intensity. Qualitatively similar dose-response relationships for the effects of Reamberin and α-LA on the behavior of the rats in the open field may

be linked with the similar effects of these drugs on insulin sensitivity [20], which plays a significant role in controlling affective-cognitive status [18]. The dose relationship of the effects of Reamberin and α-LA on behavior in the open field differed fundamentally from the corresponding relationships for their antidepressant activity (see Table 1). This does not allow changes in the rats' exploratory-orientational activity in response to Reamberin and α-LA to be interpreted solely from the point of view of their thymoanaleptic activity. The possibility that these drugs (especially at the largest doses) affect behavior in the open field also as a result of a modulatory action on the manifestation of anxiety cannot be excluded. The nature of this action can vary from anxiolytic to anxiogenic. This applies particularly to α-LA at a dose of 2EMTD, where suppressive activity on behavior in the open field may be due to both the anxiosedative effect and the "anxious freezing" reaction. Measures of behavior in the open field evidently provide insufficient information to evaluate the effects of drugs on signs of anxiety. This position is clearly illustrated by the effects of amitriptyline, whose anxiosedative action is well known. As shown in Table 1, this reference antidepressant at a dose of 2.5 mg/kg (as with the smallest dose of emoxypine and the maximum dose of Reamberin) completely prevented anxiogenic defecation in rats, though there was no effect on other measures of behavior in the open field. Complete cessation of defecation in response to amitriptyline cannot be regarded as unambiguous evidence of its tranquilizing effect because of the well-known M-cholinolytic activity of this drug, which promotes the development of constipation. The absence of any significant changes in exploratory-orientational activity in rats given amitriptyline may be due to minimization of its "behavioral toxicity" by using a relatively low dose (2.5 mg/kg). It should be noted that the smallest dose of amitriptyline used for validating the Porsolt test was 1.5 times higher – 3.75 mg/kg [19]. Use of this antidepressant at this dose produced a 25% decrease in despair behavior [19], while use at the EMTD (2.5 mg/kg) decreased immobility in rats in the Porsolt test by 48% (see Table 1). This is in good agreement with data showing a nonlinear relationship between thymoanaleptic effect and dose for antidepressants [15] and illustrates the value of using them in experimental studies at doses corresponding to the therapeutic range for humans.

Particular attention should be paid to changes in the rats' behavior in the open field after administration of Mexidol, which is simultaneously a 3-hydroxypyridine derivative and a succinic acid derivative. In contrast to isolated derivatives of 3-hydroxypyridine (emoxypine) and succinate (Reamberin), Mexidol increased exploratory activity in animals over the whole range of study doses, having no effect on orientational activity or measures of anxiety reactions (grooming and defecation) (see Table 1). Administration of Mexidol at relatively low doses (½EMTD and EMTD), as with the corresponding doses of Reamberin, induced increases in the rat's movement activity in the open field.

Standardization of the data in terms of the mean values of the corresponding controls with subsequent correlation analysis allowed the nature of the link between the antidepressant activities of the study drugs and their effects on behavior in the open field to be evaluated. This showed that the extent of the reduction in rats' despair behavior in the Porsolt test in response to study drugs increased with decreases in their stimulatory influences on the animals' orientational activity ($r_s = 0.650$, $p = 0.022$). This relationship is in quite good agreement with the higher levels of antidepressant effect with α-LA and Reamberin, the largest doses of which (in contrast to emoxypine and Mexidol) either had no effect on orientational activity in the open field (Reamberin) or suppressed it (α-LA). Evidently, the clear stimulatory component of the action of 3-hydroxypyridine derivatives (emoxypine and Mexidol) places some limit on the antidepressant activities of these drugs.

These results support the presence of antidepressant activity with emoxypine, Reamberin, Mexidol, and the reference agent (α-LA). 3-Hydroxypyridine derivatives (emoxypine and Mexidol) have lesser antidepressant effects than the succinic acid derivative (Reamberin) and α-LA, whose effects on immobility in the Porsolt test were comparable with that of amitriptyline.

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