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Comparative Analysis of the Anxiolytic Effects of 3-Hydroxypyridine and Succinic Acid Derivatives

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Threefold administration of 3-hydroxypyridine derivatives emoxipine and mexidol in optimal doses corresponding to the therapeutic dose range for humans produced an anxiolytic effect and stimulated risk behavior in the elevated plus maze test in rats. These effects were most pronounced after injection of 3-hydroxypyridine derivative emoxipine. Combination of 3-hydroxypyridine cation and succinate anion in the mexidol structure led to attenuation of the anxiolytic effect and less pronounced stimulation of the risk behavior. By the anxiolytic effect and induction of risk behavior, emoxipine and mexidol were close to the reference substance amitriptyline. Reamberin, a succinic acid derivative, had no pronounced tranquilizing properties, but risk behavior induction was similar to that produced by mexidol. In contrast to other test agents, the reference substance α-lipoic acid produced anxiogenic effects and suppressed risk behavior. The obtained results suggest that Russian-made 3-hydroxypyridine derivatives emoxipine and mexidol are promising preparations for the treatment of anxiety disorders.

Key Words: *derivatives of 3-hydroxypyridine and succinic acid; anxiolytic activity; risk behavior*

Anxiolytic substances are traditionally of high demand in the pharmaceutical market. It is related to high prevalence of anxiety disorders. Depressive disorders are also often associated with anxiety [7]. This suggests the need in substances producing simultaneously the tranquilizing and antidepressant effects. One of them, mexidol, exhibits anxiolytic and thymoanaleptic activities and can be used for the treatment of various syndromes and disorders, is one of these substances [2,4]. Mexidol is a derivative of 3-hydroxypyridine and succinic acid (SA). It can be concluded that derivatives of 3-hydroxypyridine (emoxipine) or SA (reamberin) can have similar to mexidol effects in affective disorders. This assumption was confirmed by a comparative study of the antidepressant effects of 3-hydroxypyridine and SA derivatives under experimental [3] and clinical [2,4] conditions.

Here we compared anxiolytic activities of emoxipine, reamberin, and mexidol in an experiment on rats.

MATERIALS AND METHODS

Experiments were performed on mature male and female outbred rats (n=140) weighing 180-220 g. The study was conducted in accordance with international rules regulating experiments on animals [6]. Taking into account well-known antidepressant activity of 3-hydroxypyridine and SA derivatives [3], the substances were injected in accordance to the recommendations [8] (intraperitoneally 24, 4 h, and 30 min before estimation of the anxiolytic effects). Each agent was injected in 3 doses extrapolated to single doses for

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humans taking into account the differences in relative body surface area [1]. In all cases, $^1/_2$ of the calculated equivalent of mean therapeutic dose (EMTD) served as the minimum dose and 2EMTD served as the maximum dose. We used emoxipine (Moscow Endocrine Plant) in doses of 6.25, 12.5, and 25 mg/kg, 1.5% solution of reamberin (POLISAN) in doses of 12.5, 25, and 50 ml/kg, and mexidol (Farmasoft) in doses of 12.5, 25, and 50 mg/kg. Tricyclic antidepressant amitriptyline (Amitriptyline-AKOS, Sintez) characterized by pronounced anxiolytic and sedative activities was used as the reference substance [10]. Amitriptyline was used in a dose of 2.5 mg/kg (EMTD). Additionally, we studied anxiolytic activity of α-lipoic acid (α-LA) that was previously used as the reference preparation during experimental study of antidepressant effects of 3-hydroxypyridine and SA derivatives [3]. The use of α-LA as the reference preparation was based on its sedative activity estimated previously [3]. α-LA (Berlithion; Berlin-Chemie AG/Menarini Group) was injected in doses of 25, 50, and 100 mg/kg ($^1/_2$EMTD, EMTD, and 2EMTD, respectively). All doses of the test agents were administrated in the final volume of 50 ml/kg (the substances were dissolved in 0.9% NaCl if necessary). Control rats received isotonic solution of NaCl in the same volume.

Anxiolytic activity of the substances was estimated by their effects on rat behavior in the elevated plus maze (EPM) made according to the description [9]: it consisted on two stainless steel bands placed perpendicularly to each other and forming four arms of EPM (60×12 cm each) and central area (12×12 cm). Two opposite arms (50 cm) of the EPM had walls (closed arms) and the other (open arms) had no walls. The EPM was positioned on a stainless steel support leg (diameter 4.8 cm) at a height of 50 cm above the floor.

At the beginning of the test, the rat was placed to the central area facing to one of open arms. The number of entries into open and closed arms and central area was recorded over 5 min. The total time spent in these parts of EPM was also recorded. The obtained results were presented as absolute levels and percentage of the total number of entries into EPM arms and time spent there (of total testing time). The animal was suggested to enter EPM area when all its paws were in an arm or in the central area. The total motor activity of animals (total number of entries into various EPM areas) and risk behavior were also registered. Risk behavior was estimated by the number of head-dipping postures (animal head below the EPM floor), number of entries into terminal parts of open arms (10 cm from the edge), and number of stretching (a rat actively stretched while location of its hind legs remained unchanged).

In accordance to generally accepted recommendations [11], the level of anxiety was estimated by the number of entries into closed arms of EPM and time spent there. The number of entries into open arms and central area of the EPM and total time spent there were considered as parameters of anxiolytic effects of substances.

Statistical analysis of the obtained results was performed using SPSS 13.0 software. The data were analyzed by methods of descriptive statistics and presented as median (Me) and lower (Q25) and higher (Q75) quartiles. Between-group differences were evaluated using Mann–Whitney *U* test. The correlations were calculated using Spearman correlation coefficient (r_s). Estimation of statistical hypothesis was performed at critical significance level p=0.05.

RESULTS

All studied 3-hydroxypyridine and SA derivatives significantly increased the total number of entries into the central area of the EPM (Table 1). This effect was typical of all studied doses of emoxipine, reamberin, and mexidol. The reference substances (amitriptyline in a dose of 2.5 mg/kg and α-LA in the minimum and medium doses) had similar effects. Along with the increase in the number of entries into the central area, 3-hydroxypyridine and SA derivatives increased the total number of entries into open arms of the EPM (Table 1). This effect was observed after treatment with relatively high doses of emoxipine (EMTD and 2EMTD) and medium doses (EMTD) of reamberin and mexidol. None of the reference substances produced this effect.

As commonly accepted, the increase in the number of entries into open arms and central area of the EPM after treatment attests to the tranquilizing effect of the test drugs [5,11]. However, these effects of 3-hydroxypyridine and SA derivatives cannot be clearly explained by an increase in the number of entries into closed arms of the EPM (Table 1), which is considered as a sign of anxiety [11]. This effect was observed after treatment with relatively high doses of emoxipine and reamberin, and minimum dose of mexidol and α-LA. Amitriptyline in a dose of 2.5 mg/kg produced no such effects. These findings suggest that the increase in the number of transfers between EPM parts after treatment with the test substances reflect their ability to enhance total motor activity of animals. This hypothesis was confirmed by a significant increase in the integral parameter of locomotor activity of rats receiving emoxipine and mexidol in all doses, and reamberin in relatively high doses (EMTD and 2EMTD; Table 1). Amitriptyline in a dose of 2.5 mg/kg and α-LA in relatively low doses ($^1/_2$EMTD and EMTD)

TABLE 1. Effects of 3-Hydroxypyridine and SA Derivatives, Amitriptyline, and α-LA on the Number of Entries into EPM Areas

Group	Entries into closed arms		Entries into open arms		Entries into central area		Total locomotor activity, abs.
	abs.	%	abs.	%	abs.	%	
Control (*n*=10)	5.0 (2.5-7.3)	44.4 (31.2-49.6)	2.0 (1.0-3.3)	21.4 (10.6-30.2)	3.5 (1.8-7.5)	43.7 (22.3-47.5)	10.5 (6.5-15.8)
Amitriptyline (2.5 mg/kg; *n*=10)	6.0 (4.8-9.0)	33.4 (28.9-37.6)	3.5 (2.8-6.0)	21.1 (15.2-24.7)	9.5* (5.5-12.5)	47.0 (44.0-48.1)	20.0* (13.0-26.0)
Emoxypine							
1/EMTD (6.25 mg/kg; *n*=10)	6.5 (5.8-8.3)	37.2 (33.3-39.3)	3.0 (3.0-4.0)	17.4 (14.3-21.7)	9.0* (6.5-10.3)	47.0 (42.4-47.7)	20.0* (15.0-22.3)
EMTD (12.5 mg/kg; *n*=10)	8.0* (7.5-9.3)	32.7 (31.9-38.6)	5.0* (3.8-6.0)	20.0 (16.5-24.0)	11.5* (9.8-13.0)	48.0 (46.5-48.2)	25.0* (20.8-27.0)
2EMTD (25 mg/kg; *n*=10)	8.0* (6.8-9.3)	36.2 (31.5-38.3)	4.5* (3.8-5.3)	19.7 (16.6-23.1)	10.5* (7.8-13.0)	47.7 (41.0-48.2)	23.5* (18.0-27.0)
Reamberin							
1/2EMTD (12.5 ml/kg; *n*=10)	6.0 (5.0-7.0)	39.0 (32.5-45.6)	2.0 (1.0-4.3)	14.3 (8.7-18.8)	7.5* (5.8-9.5)	46.4 (45.3-47.5)	16.0 (12.5-20.8)
EMTD (25 ml/kg; *n*=10)	7.5* (7.0-9.0)	31.1 (28.8-38.0)	5.0* (4.0-6.3)	22.0 (17.3-23.0)	12.5* (9.8-13.3)	48.1 (46.4-48.2)	26.0* (21.8-27.5)
2EMTD (50 ml/kg; *n*=10)	7.0* (7.0-8.3)	37.5 (33.3-41.2)	3.5 (2.8-4.0)	16.8 (14.1-19.5)	9.5* (6.8-10.5)	47.5 (40.9-47.7)	20.0* (16.5-22.0)
Mexidol							
1/2EMTD (12.5 mg/kg; *n*=10)	6.5* (6.0-9.0)	36.4 (31.1-48.2)	3.0 (1.0-5.0)	16.8 (3.8-21.0)	7.5* (6.8-10.5)	45.8 (38.4-47.7)	20.0* (16.8-25.0)
EMTD (25 mg/kg; *n*=10)	7.0 (6.0-8.5)	32.7* (31.3-34.5)	5.0* (3.8-5.0)	21.4 (17.2-24.0)	10.5* (8.5-13.3)	47.9 (43.8-48.3)	22.5* (18.8-27.5)
2EMTD (50 mg/kg; *n*=10)	7.0 (4.8-8.0)	34.1 (28.8-43.3)	4.0 (2.0-5.3)	18.2 (12.6-23.2)	9.5* (8.3-10.3)	47.6* (47.4-47.9)	20.0* (18.0-21.5)
α-LA							
1/2EMTD (25 mg/kg; *n*=10)	8.0* (6.8-8.3)	36.7 (32.5-42.3)	3.0 (2.8-6.0)	17.9 (14.3-20.6)	9.5* (8.8-13.5)	47.6* (46.5-48.9)	20.5* (15.3-27.8)
EMTD (50 mg/kg; *n*=10)	6.0 (5.0-7.3)	37.7 (34.1-43.1)	2.5 (2.0-4.0)	15.6 (11.5-20.3)	8.5* (6.0-9.0)	46.6 (45.9-47.4)	18.0* (13.0-19.3)
2EMTD (100 mg/kg; *n*=10)	4.0 (2.8-4.8)	36.6 (32.7-44.6)	2.0 (1.0-3.0)	17.0 (13.8-20.1)	4.0 (3.0-6.8)	45.5 (42.9-47.4)	9.5 (7.0-14.5)

Note. Entries of animals into various parts of the EPM are presented as absolute levels and percent of total locomotor activity of animals. Here and in Table 2: *$p<0.05$ in comparison with the control.

TABLE 2. Effects of 3-Hydroxypyridine and SA Derivatives, Amitriptyline, and α-LA on Parameters of Risk Behavior in the EPM

Group	Stretching[1]			Head-dipping postures				Entries into the terminal parts of open arms
	closed arms	opened arms	total number	closed arms	opened arms	central ground	total number	
Control (*n*=10)	2.0 (1.8-2.0)	2.0 (0.8-2.0)	3.0 (2.8-4.0)	2.0 (1.0-2.3)	1.0 (0.8-3.3)	2.0 (1.0-3.3)	4.5 (3.0-8.5)	0 (0-2.0)
Amitriptyline (2.5 mg/kg; *n*=10)	1.5 (1.0-2.0)	2.0 (1.8-3.3)	3.5 (2.8-5.3)	2.0 (1.0-2.3)	2.5 (1.8-6.0)	3.0 (1.0-3.3)	7.5 (3.8-10.5)	2.0* (1.5-4.0)
Emoxypine								
$^1/_2$EMTD (6.25 mg/kg; *n*=10)	2.0 (2.0-2.0)	1.5 (0-2.8)	3.5 (2.0-5.0)	2.0 (2.0-2.0)	2.0 (1.0-4.0)	2.0 (1.8-3.0)	6.0 (4.8-9.0)	2.5* (1.8-3.0)
EMTD (12.5 mg/kg; *n*=10)	2.0 (2.0-2.0)	2.0 (1.0-3.5)	4.0 (3.0-6.3)	2.0 (2.0-3.3)	4.5* (1.5-5.3)	4.0* (3.0-5.0)	11.0* (7.8-12.0)	3.0* (1.8-4.0)
2EMTD (25 mg/kg; *n*=10)	2.0 (2.0-2.3)	2.0 (1.0-3.3)	4.0* (3.8-6.0)	2.0 (2.0-3.0)	2.5* (2.0-6.0)	3.0 (2.0-3.3)	8.5* (7.0-10.5)	2.0* (1.8-3.0)
Reamberin								
$^1/_2$EMTD (12.5 ml/kg; *n*=10)	2.0 (1.8-2.0)	1.5 (0-4.0)	3.5 (2.0-5.3)	2.0 (1.8-3.0)	0.5 (0-3.0)	3.0 (1.8-4.0)	5.5 (3.0-9.0)	1.0 (0-2.5)
EMTD (25 ml/kg; *n*=10)	2.0 (1.0-2.3)	2.0 (0.8-4.0)	4.5 (2.0-5.3)	2.5 (2.0-4.0)	3.0 (1.8-5.3)	3.5* (2.8-5.0)	10.0* (6.5-12.5)	2.5* (1.0-3.0)
2EMTD (50 ml/kg; *n*=10)	2.0 (2.0-3.0)	2.5 (1.8-3.3)	5.0* (4.0-5.3)	2.5 (2.0-3.0)	2.5 (0.8-4.3)	2.0 (2.0-3.3)	7.0 (5.0-11.5)	2.0* (1.8-3.0)
Mexidol								
$^1/_2$EMTD (12.5 mg/kg; *n*=10)	1.5 (1.0-2.0)	0.5 (0-4.0)	2.0 (1.0-5.3)	2.0 (1.8-3.0)	1.0 (0-4.3)	3.0 (1.8-3.0)	6.5 (4.0-9.3)	1.5 (0-2.0)
EMTD (25 mg/kg; *n*=10)	2.0 (2.0-3.3)	2.0 (1.8-4.0)	4.5* (3.8-8.0)	2.0 (1.8-3.3)	3.0 (1.8-6.0)	2.5 (1.8-3.5)	10.0* (7.3-10.0)	2.5* (1.0-4.0)
2EMTD (50 mg/kg; *n*=10)	2.0 (1.8-2.3)	2.0 (1.5-4.0)	4.5 (3.3-6.0)	2.0 (2.0-3.0)	1.5 (0.8-3.5)	3.0 (2.0-4.0)	8.0 (6.3-9.0)	2.0* (1.8-3.3)
α-LA								
$^1/_2$EMTD (25 mg/kg; *n*=10)	2.0 (1.0-2.3)	1.5 (0-4.0)	3.5 (2.0-6.3)	2.0 (2.0-3.0)	1.0 (0.8-3.0)	3.0 (1.8-4.0)	7.0 (4.0-9.0)	1.5 (0.8-3.3)
EMTD (50 mg/kg; *n*=10)	1.0 (1.0-2.0)	2.0 (0-3.0)	3.0 (1.8-4.3)	2.0 (2.0-2.3)	1.5 (0.8-4.0)	2.0 (1.0-2.3)	6.0 (3.8-8.3)	1.5 (0-2.3)
2EMTD (100 mg/kg; *n*=10)	1.0* (0-1.3)	1.0 (0.8-2.0)	2.0* (1.0-3.0)	1.0 (1.0-2.0)	1.0 (0-2.3)	1.0 (0-2.3)	3.5 (2.0-5.3)	1.0 (0-2.3)

Note. Absolute parameters of risk behavior of rats over 5 min are presented. [1]Stretching was registered only in arms of the EPM.

also promoted an increase in locomotor activity of animals.

Against the background of increased motor activity of animals treated with the test substances, the most reliable parameter for evaluation of their tranquilizing effects is the time spent in various parts of EPM. This parameter changed only after administration of emoxipine and α-LA in maximum doses. Under these conditions emoxipine revealed pronounced anxiolytic effect and significantly decreased absolute (from 193 (162-212) sec in control to 153.0 (143.3-170.5) sec; p=0.015) and relative (from 64.3 (54.0-70.7)% in control to 51 (47.7-56.8)%; p=0.015) time spent in closed arms of the EPM and increased time spent in open arms (from 47.5 (34.3-59.3) sec in control to 59.5 (53.2-82.8) sec; p=0.028; from 15.8 (11.4-19.7)% in control to 19.8 (17.8-27.6)%; p=0.028, respectively). α-LA had anxiogenic properties and significantly reduced absolute (from 66 (47-85) sec in the control to 39.5 (13.8-43.8) sec; p=0.014) and relative (from 22.0 (15.7-28.3)% in the control to 13.2 (4.6-14.6)%; p=0.014) time spent in the central area of the EPM. In control group rats, the negative correlation between the relative time spent in closed arms and central area (r_s=-0.81, p=0.005) was more pronounced than the correlation between the relative time spent in closed and open arms (r_s=-0.689, p=0.028). These data suggest that not only time spent in the open arms, but also time spent in the central area of EPM is a conclusive criterion for estimation of anxiety suppression.

Reamberin, mexidol, and amitriptyline did not affect the time spent in various parts of EPM. However, mexidol, in contrast to reamberin, significantly reduced the number of entries into closed arms (EMTD) and increased the relative number of entries into the central area (maximum dose, 2EMTD; Table 1). These results led us to a conclusion that mexidol has a mild tranquilizing effect, but its anxiolytic activity is significantly lower than the corresponding activity of emoxipine. Administration of α-LA in the minimum dose (as distinct from 2EMTD) also had mild anxiolytic effect and induced a significant increase in the relative number of entries into the central area of the EPM. Amitriptyline and reamberin did not affect percent ratio of entries into various parts of the EPM.

The effects of 3-hydroxypyridine and SA derivatives on risk behavior were similar to their effects on the preference of various parts of the EPM (Table 2). At a first glance, it indicates that risk behavior is an indirect manifestation of anxiolytic activity. However, the results of the correlation analysis performed in the control group confirmed this hypothesis only for the number of head-dipping from open arms. This factor was the only parameter of risk behavior, which significantly directly correlated with the relative time spent in open arms (r_s=0.677, p=0.032).

Emoxipine induced the most pronounced increase in the parameters of risk behavior. It enhanced the number of entries into the terminal parts of open arms (in all doses), number of head-dipping from the open arms of EPM and total number of head-dipping postures (in relatively high doses), the number of head-dipping postures from the central area (EMTD), and total number of stretching (2EMTD).

In contrast to emoxipine, mexidol in the medium dose (EMTD) did not affect the number of head-dipping postures from the open arms, but significantly increased the total number of head-dipping postures. Administration of mexidol in this dose also induced an increase in the total number of stretching. Treatment with mexidol in relatively high doses (EMTD and 2EMTD) promoted an increase in the number of entries into the terminal parts of open arms. These findings agree with the data that the anxiolytic effect of mexidol is inferior to that of emoxipine.

The effects of reamberin on parameters of risk behavior were similar to the effects of mexidol. Reamberin in relatively high doses increased the number of entries into the terminal parts of open arms. Administration of this agent in the medium dose increased the number of head-dipping postures from the central area and total number of head-dipping postures and reamberin in maximum dose increased the total number of stretching.

The effects of amitriptyline in the dose of 2.5 mg/kg on risk behavior were less pronounced than the effects of 3-hydroxypyridine and SA derivatives. This antidepressant with anxiolytic and sedative activity induced an increase in the number of entries into the terminal parts of open arms, but did not affect the number of head-dipping and stretching postures. As distinct from other agents, α-LA suppressed the risk behavior in the EPM. It manifested in a significant decrease in the number of stretching in closed arms and total number of stretching after injection of α-LA in the maximum dose. These data confirm the presence of anxiogenic activity of α-LA in 2EMTD dose.

The obtained results suggest that anxiolytic activity of 3-hydroxypyridine and SA derivatives and their ability to promote risk behavior mostly depend on the presence of 3-hydroxypyridine residue in their structure. This suggestion is confirmed by the most significant tranquilizing effect of 3-hydroxypyridine derivative (emoxipine), which also induced the most significant increase in risk behavior. Combination of hydroxypyridine cation and succinate anion in mexidol structure led to attenuation of the anxiolytic effect and less pronounced stimulation of risk behavior. SA derivative (reamberin) had no pronounced tranquilizing

effect, but stimulated risk behavior (similar to mexidol). The obtained results reflect the prospects of using Russian 3-hydroxypyridine derivatives emoxipine and mexidol for treatment of anxiety disorders.

REFERENCES

1. I. A. Volchegorskii, I. I. Dolgushin, O. L. Kolesnikov, and V. E. Tseilikman, *Experimental Modeling and Laboratory Estimation of Human Adaptive Reactions* [in Russian], Chelyabinsk (2000).
2. I. A. Volchegorskii, M. G. Moskvicheva, and E. N. Chashchina, *Klin. Med.*, **82**, No. 11, 31-35 (2004).
3. I. A. Volchegorskii, I. Yu. Miroshnichenko, L. M. Rassokhina, and R. M. Faizullin, *Eksp. Klin. Farmakol.*, **76**, No. 7, 6-10 (2013).
4. I. A. Volchegorskii, E. V. Pravdin, and T. V. Uzlova, *Bull. Exp. Biol. Med.*, **156**, No. 3, 347-352 (2014).
5. A. E. Grigor'eva, D. A. Smagin, N. P. Bondar', *et al.*, *Zh. Vyssh. Nervn. Deyat.*, **63**, No. 4, 486-494 (2013).
6. R. A. Kopaladze, *Uspekhi Fiziol. Nauk*, **29**, No. 4, 74-92 (1998).
7. A. B. Smulevich, *Depressions during Somatic and Psychical Disorders* [in Russian], Moscow (2003).
8. J. J. Buccafusco, *Methods of Behavioral Analysis in Neuroscience*, London; New York (2009).
9. A. Loche, F. Simonetti, C. Lobina, *et al.*, *Front. Psychiatry*, **3**, No. 8, doi: 10.3389/fpsyt.2012.00008 (2012).
10. E. J. Nestler, S. E. Hyman, R. S. Malenka, *Molecular Neuropharmacology: a Foundation for Clinical Neuroscience*, New York (2001).
11. A. A. Walf, and C. A. Frye, *Nat. Protoc.*, **2**, No. 2, 322-328 (2007).