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The Antidepressant-Like Activity Of The New Tetrapeptide Neuroprotector Kk-1, Homologous Of $Acth_{15-18}$ Sequence (An Experimental Study)

Roman D. Deiko[1*], Sergiy Yu. Shtrygol[2], Alexandr A. Kolobov[3] , Andrey S. Simbirtsev[4] Maria V. Mishchenko[5]

1. Post-graduate student of the department of pharmacology of National University of Pharmacy

2. The head of the department of pharmacology of National University of Pharmacy

3. The head of peptide chemistry laboratory of State Research Institute of Highly Pure Biopreparations of Russian Federal Medico-Biological Agency

4. Director of State Research Institute of Highly Pure Biopreparations of Russian Federal Medico-Biological Agency

5. 4th year student of National University of Pharmacy

Abstract.

The aim of study is to evaluate antidepressant-like activity of the new peptidergic neuroprotector acetyl-(D-Lys) -Lys-Arg-Arg-amide, homologous of $ACTH_{15-18}$ primary amino acids sequence, that demonstrates nootropic and neuroprotective properties.

Using Porsolt swimming test (PST) efficacy of tetrapeptide neuroprotector KK-1 at a single dose of 0.02 mg/kg was investigated on 16 white random bred male rats (body mass equaled 180-220 grams). Imipramine (15 mg/kg i.p.) was used as a reference drug. Then depression was induced in these rats by reserpine (4 mg/kg, i.p.). The KK-1 (intranasally, i.n.) and imipramine were administered once a day during 3 days until the reserpine-induced depression was reproduced. The indices of rats behavior under the conditions of open-field test (OFT) and PST were evaluated. The influence of both drugs on specific reserpine-induced depressions symptoms (hypothermia and blepharoptosis) was also registered. The results were processed statistically.

The tetrapeptide neuroprotector KK-1 reduced immobilization time of rats at PST (statistically significant differences compared with control group), exceeding efficacy of reference drug imipramine. Normalizing of locomotor and exploratory activity in the OFT, decreasing indices of rats helplessness behavior in PST by tetrapeptide neuroprotector KK-1 demonstrates its antagonism with depressive action of reserpine. The tetrapeptide KK-1 showed antidepressant-like action both in intact rats and in rats with the model of reserpine-induced depression. It reduced specific symptoms of depression – hypothermia and blepharoptosis, exceeding the activity of reference drug imipramine.

Corresponding author: Master of Pharmacy, post-graduate student of the department of pharmacology of National University of Pharmacy. Address: Ukraine, Kharkiv, Kulykivska-street, 12. Tel. +380 99 962 13 83,

email: roman.deyko@gmail.com

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Introduction.

Organic and functional disorders of CNS are widespread in population and often are accompanied with depressive symptomatic [1]. The much of these cases neuroprotective therapy are needed. The neuroprotectors are relatively new pharmacological class of drugs improving CNS function and protecting neurons from damaging factors [2]. Peptidergic substances are perspective group of neuroprotectors. Adrenocorticotropine derivatives, brain-derived neurotrophic factor as well as drugs developed on their basis have pleiotropic mechanism of protective action and demonstrate advantageous psychotropic properties.

The depressive disorders are determined largely by monoaminergic transmission deficit and are seen at bipolar disorder and numerous CNS disorders of diverse etiology [3]. Numerous CNS disorders such as acute cerebrovascular diseases are followed by secondary brain tissue injuries and impairment of patient mental health. In clinical picture of vascular and traumatic brain injuries the depressive symptomatic often take a place [1]. The neuroprotectors with pleiotropic mechanism of action influencing on several neuromediator systems of the brain are perspective as depressive disorders correctors.

The literature data indicate frequent neuroprotectors using at comorbid CNS pathology [4,5]. A natural relation between ability of numerous psychotropic drugs to modulate the high mental functions and to protect neurons from damage is known. Thus as the some nootropic drugs from racetams class (fonturacetam, pramiracetam) are often used like anti-asthenic and antidepressant-like medicines [6,7]. Furthermore, the neuroprotective properties of these drugs at cerebrovascular and neurodegenerative diseases are well known [8].

It's well known that many neuropeptides [9, 10], as well as drugs created on their basis [11,12] show the antidepressant-like properties under the conditions of experimental or clinical trials. In the recent literature data the reviews of psychopharmacologic profile of many peptide neuroprotectors are presented.

Our attention was attracted by series of new peptidergic neuroprotectors. In the basis of their structure underlies the primary amino acids sequence of adrenocorticotropine 15-18 (Lys-Lys-Arg-Arg, $ACTH_{15-18}$). As it's known from the literature data, $ACTH_{15-18}$ is minimal fragment of ACTH capable to bind with melanocortin receptors (MCRs), that plays role of antagonist of the cerebral and peripheral MCRs [13]. Moreover, the sequence Lys-Lys-Arg-Arg is presented in the structures of some neurotrophic factors, such as nerve growth factor and brain-derived neurotrophic factor [14]. Taking into account the physiologically role of $ACTH_{15-18}$, on its basis at the State Research Institute of Highly Pure Biopreparations (Russia, Saint-Petersburg) was synthetized the series of modified tetrapeptides with general formula acetyl-Lys-Lys-Arg-Arg-amide [15]. The some amino acids were inserted in tetrapeptide chain as unnaturally D-forms (D-Lys or/and D-Arg). It has improved their resistance to the blood peptidases action and allowed to administrate these drugs only once a day. Subsequently under the conditions of pre-clinical studying were established their pronounced neuroprotective and nootropic properties [16,17].The leader among 10 investigated peptides according to the neuroprotective, anti-hypoxic, nootropic, anti-alcoholic and other protective properties is tetrapeptide KK-1 (acetyl-(D-Lys)-Lys-Arg-Arg-amide).

Since, the tetrapeptide KK-1 may function in CNS as an ACTH antagonist, it is appropriate to suppose the antidepressant-like properties of its. Earlier we have tested KK-1 on mice in the tail suspension test [18]. There was shown the absence of influence of KK-1 at the dose of 0.02 mg/kg on the general time of immobilization of mice. But this test is considered to be less sensitive than Porsolt swimming test (PST). Furthermore, the different sensitivity of animals' species (rats and mice) to the expected antidepressant-like action of neuropeptide KK-1 should not to be excluded.

The aim of the study is to find out the efficacy of new tetrapeptide neuroprotector KK-1 on the model of reserpine-induced depression in rats.

Experimental Procedures.

The experiment was carried on 16 white random bred sexually mature male rats weighing 180-220 g. The animals were obtained from the vivarium of Central Research Laboratory of NUPh. All pharmacological tests were performed following the requirements of the «General Ethical Principles for Experiments on Animals» (Kyiv, 2001) harmonized with the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purpose" (Strasbourg, 1986).

The antidepressant-like activity of tetrapeptide neuroprotector KK-1 administered at a dose of 0.02 mg/

kg intranasally (i.n.) was evaluated. The dose and route of administration of tetrapeptide KK-1 were chosen according to our previously studies, discovered neuroprotective and nootropic properties of this drug. 5-10μl of aqueous solution of KK-1 were carefully placed on one nostril of conscious animal, allowing it to be snorted, alternating the nostrils with 1 min intervals. As reference drug was used classical tricyclic antidepressant imipramine (Melipramine, EGIS, Hungary) at a dose of 15 mg/kg intraperitoneal (i.p.). The experiment was performed in 2 stages. In the 1st stage the animals were divided into the groups as follows: 1 – control group (n=5), 2 – imipramine group (15 mg/kg i.p., n=6), 3 – KK-1 group (0.02 mg/kg i.n, n=5). 25-30 min before experiment beginning the measuring of rats' rectal temperature and administration of the appropriate drug were done. The control group animals received 0.9% saline i.p. and i.n. Then the locomotor activity (number of crossed squares) and exploratory activity (number of vertical uprights and surveys holes), emotional reactions and their vegetative accompaniment (fecal boli, urinations and grooming acts) at open-field test (OFT) for rats were registered for 3 min [19, 20]. The animals depressivity indices (latent time of immobilization, number of immobilizations acts and their general time) were evaluated at PST for 6 min (24 hours before testing at PST the rats were pretested under the same conditions for 15 min) [20, 21]. During the next 2 days the medicines were administered at the same doses once a day, last time – 30 min before reserpine administration. Reserpine (Sigma, USA) was administered *per os* on the 3rd day (2nd stage of experiment) at a dose of 4 mg/kg as suspension stabilized with tween-80. After 6-7 h behavioral indices of all experimental groups were evaluated at OFT and PST. The rectal temperature was measured with electronic thermometer Microlife MT 1951 (Switzerland), the blepharoptosis degree was rated using 4-point scored scale (from 1 to 4) [19] and relative quantify (%) of animals in the group with maximally expressed blepharoptosis.

Statistical analysis was performed using one-way analysis of variances. Fisher angular transformation of φ was used when the results were determined in alternative manner (presence or absence of characteristic). The significance of intragroup differences were determined by Ť-Wilcoxon's criterion. Inter- and intragroup differences were considered statistically significant at $p<0.05$.

Results.

The results of OFT are presented in table 1.

As seen from the table 1, the investigated tetrapeptide KK-1 decreased locomotor and exploratory activity of intact animals in OFT as well as reduced their emotional reactions. Imipramine showed a tendency to decrease these activity markers of the intact animals. The level of their emotional reactions was increased under the conditions of imipramine administration (in 2.9 times, $p<0.05$ compared with control group).

Reserpine induced strongly pronounced depression (see table 1) which is characterized by the reduction of the locomotor activity (by 70%, $p<0.05$ comparing with index of intact animals), exploratory activity values (by 93.2%, $p<0.05$ comparing with index of intact animals), and by tendency to increment in emotional reactions level in 1.9 times. The sum of all indices at OFT was decreased by 97% ($p<0.05$). Under the conditions of imipramine use the level of these disorders fractionally decreased. Locomotor activity was reduced by 79.2% ($p<0.05$ compared with index of intact animals), exploratory activity was decreased by 76.5% ($p<0.05$ compared with value of intact animals). Emotional reactions level decreased not significantly in 1.6 times ($p>0.05$ compared with index of intact animals), but the sum of all indices at OFT decreased, although not to the such degree as in control group, but statistically significant (by 69.2%, $p<0.05$). Decreasing of locomotor and exploratory activities of animals treated with tetrapeptide KK-1 is less pronounced comparing with control and imipramine groups. The number of crossed squares was decreased only as tendency by 15 % and exploratory activity – by 20.7% ($p>0.05$ compared with value of intact animals). The sum of all indices was decreased under the conditions of KK-1 administration by 29.3% that was statistically insignificant ($p>0.05$ compared with index of intact animals). Only the sum of emotional reactions and their vegetative support decreased significantly in 9 times ($p<0.05$ compared with value of intact animals), while in the untreated animals these conversely increased.

As shown in table 2, according to the results of PST tetrapeptide neuroprotector KK-1 demonstrated antidepressant-like properties both in intact animals and in the animals with reserpine-induced depression.

The antidepressant-like properties of

Table 1. Influence of peptide neuroprotector KK-1 and reference drug imipramine on the indices of intact animals and animals with reserpine-induced depression in open-field test

Indices	The condition of experiment	Group, n		
		Control, n=5	Imipramine, n=6	KK-1, n=5
Locomotor activity (number of squares)	I	18.4±4.79	9.6±1.53	4.0±1.34*^
	R	4.4±0.87 (– 70.0%)®	2.0±0.55* (– 79.17%)®	3.4±2.4 (– 15.0%)
Exploratory activity (the sum of rearings and nose-pokes)	I	13.2±3.64	6,8±0,77	5,8±0,77*
	R	2,6±1,63 (– 93.18%)®	1.6±0.4 (– 76.47%)®	4.6±2.4 (– 20.69%)
Emotional reactions and vegetative accompaniment of these (the sum of fecal boli, urinations and grooming acts)	I	1.6±0.58	4.6±0.58*	1.8±0.77^
	R	3.0±0.55 (+ 87.5%)	2.8±1.39 (– 39.12%)	0.2±0.2* (– 88.9%)®
The sum of all indices	I	33.2±8.43	20.8±2.68	11.6±2.49*^
	R	10.0±1.30 (– 97.0%)®	6.4±1.6* (– 69.23%)®	8.2±4.98 (– 29.3%)

Note. *I* – intact animals, *R* – reserpine-depressed animals; p≤0.05: * – compared with control group, ^ – compared with imipramine group, ® – between the indices of the same rats before and after reserpine-induced depression. The changes of indices (in %) of the same rats before and after reserpine-induced depression are given in brackets.

tetrapeptide neuroprotector KK-1 in intact animals consists in its ability to increase the latent time of immobilization that is statistically significantly (in 3.5 times comparing with control group, $p<0.05$), to decrease the number of immobilizations episodes and their durations time (in 11.5 and 16.1 times respectively, $p<0.05$ compared with control group). The imipramine treated animals were characterized only by decreasing of the number of immobilizations episodes, that was statistically significantly ($p<0.05$ compared with control group), but the other indices (latent time of immobilization and general time of immobilizations) changed only as tendency.

Under the conditions of reserpine-induced depression neuropeptide KK-1 also demonstrated statistically significant antidepressant-like effect. As in case with intact animals, KK-1 increased latent time of immobilization in 4.5 times ($p<0.05$ compared with control group), decreased number of immobilizations episodes in 2.6 times ($p<0.05$ compared with control group) and general time of immobilizations in 3.6 times ($p<0.05$ compared with control group).

The influence of the studied drugs on the rats' rectal temperature is shown in figure 1.

As seen from figure 1, the basic temperature level of rats of all 3 groups is comparable and corresponds to normal index of these animals' species. The rats' rectal temperature did not change under the influence of administered compounds (the 2nd column of histogram). Reserpine at a dose of 4 mg/kg in 6 hours acutely decreased rectal temperature of rats in all investigated groups. Only in control group the value of temperature was decreased statistically significant ($p<0.05$). In KK-1 group it was less expressed than in control group and in imipramine group it was minimal ($p>0.05$ compared with control group). Thus, both investigated drugs, tetrapeptide neuroprotector KK-1 and reference drug imipramine demonstrated a tendency to attenuation of hypothermia – typical reserpine-induced depression symptom.

Blepharoptosis is also among the symptoms of the depression developed.

Table 2. Influence of tetrapeptide neuroprotector KK-1 and reference drug imipramine on the indices of intact animals and animals with reserpine-induced depression under the conditions of Porsolt swimming test

Indices	The condition of experiment	Group, n		
		Control, n=5	Imipramine,	KK-1, n=5
Latent time of immobilizations, sec	I	61.8±18.5	106.8±25.0	214.6±66.7*
	R	38.2±17.2 (– 39.2%)	128.8±44.5 (+ 20.6%)	170.8±34.1* (– 20.4%)
Number of immobilizations episodes	I	13.8±3.08	5.8±1.6*	1.2±0.6*
	R	7.8±0.96 (– 43.5%)	5.2±1.13 (– 10.3%)	3.0±0.38* (+ 150.0%)
General time of immobilizations, sec	I	64.4±32.5	18.0±6.9	4.0±2.7*
	R	28.2±8.1 (– 56.2%)	17.7±5.8 (– 1.7%)	7.8±2.3* (+ 95.0%)

Note. *I* – intact animals, *R* – the animals with reserpine-induced depression; * – p≤0.05 compared with control group; The changes of indices (in %) of the same rats before and after reserpine-induced depression are given in brackets.

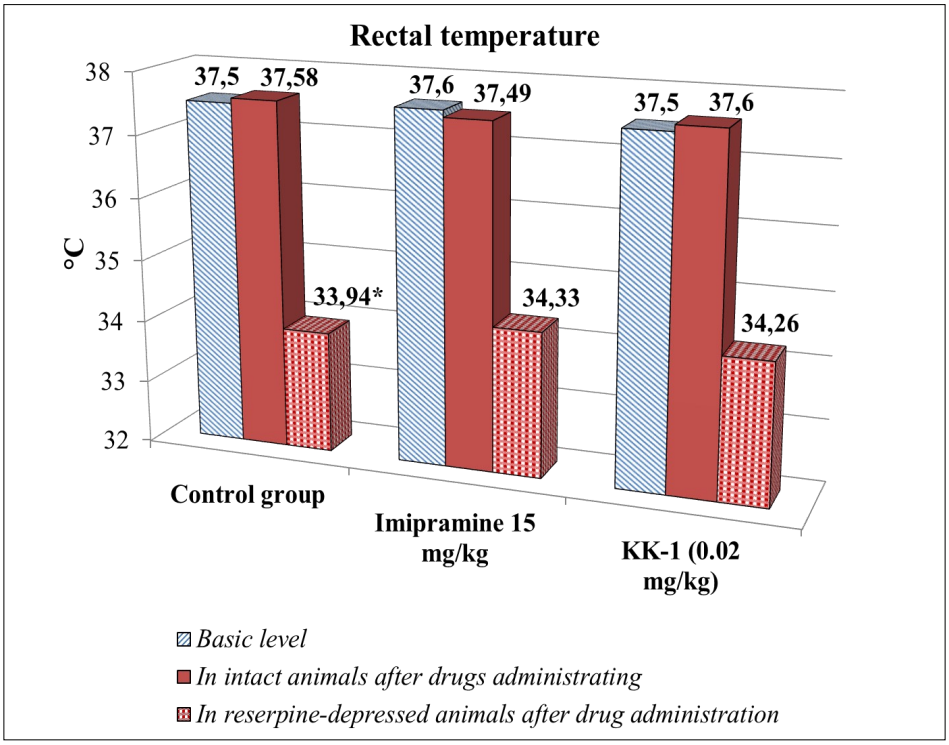

Figure 1. Influence of tetrapeptide neuroprotector KK-1(0.02 mg/kg i.n.) and reference drug imipramine (15 mg/kg i.p.) on the rats with reserpine-induced depression rectal temperature. Note: * – p≤0.05 compared with basic level of temperature of the same rats.

As seen from figure 2, both investigated drugs decreased expression of blepharoptosis, that was statistically significant. Under the conditions of the treatment with imipramine this index decreased by 38.9% ($p<0.05$ compared with control group) and with tetrapeptide neuroprotector KK-1 – it decreased by 33.3% ($p<0.05$ compared with control group).

The number of animals with maximally pronounced blepharoptosis (4 points – eyes are totally closed) in each group is represented in figure 3.

As seen from figure 3, the number of animals with maximally pronounced blepharoptosis in control group was 60%. Imipramine showed a tendency to decreasing of this index by 26.7%. In KK-1 group, the animals with maximally expressed blepharoptosis were completely absent ($p<0.05$ compared with control group). It confirms antagonistic interaction of the new tetrapeptide neuroprotector KK-1 with depressogenic action of reserpine.

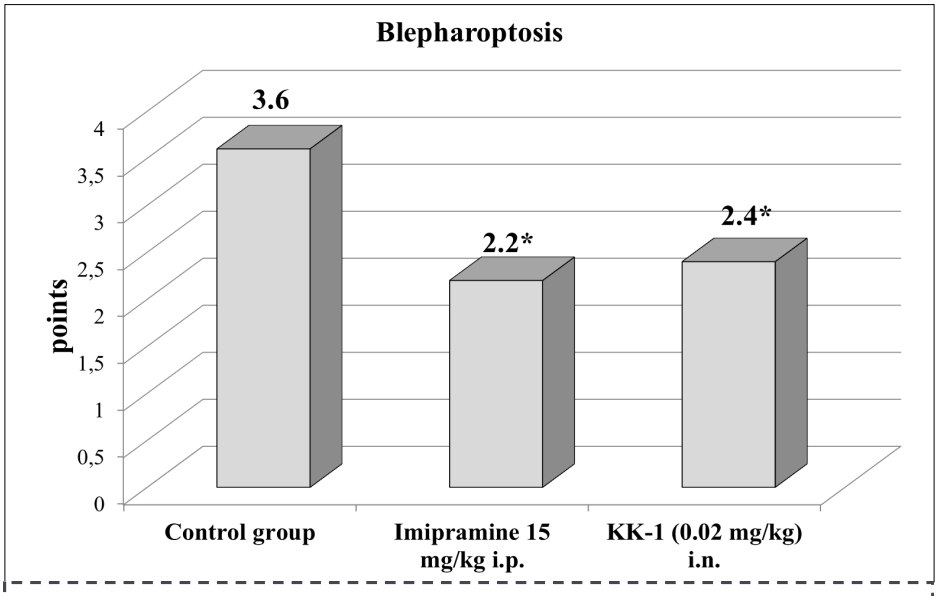

Figure 2. Influence of tetrapeptide neuroprotector KK-1 at a dose of 0.02 mg/kg i.n. and reference drug imipramine (15 mg/kg i.p.) on the rats with reserpine-induced depression blepharoptosis. *Note*: * – p≤0.05 with control group

Discussions.

The investigated object was the neuroactive tetrapeptide, homologous of $ACTH_{15-18}$ amino acids sequence, with chemical structure acetyl-(D-Lys)-Lys-Arg-Arg-amide (laboratory code KK-1). Under the conditions of experimental studies it demonstrated pronounced neuroprotective and advantageous psychotropic properties [16, 17, 18]. Since tetrapeptide KK-1 is a highly active pharmacological agent, an investigation of its antidepressant-like properties was needed. We used OFT and PST for the evaluation of tetrapeptide KK-1 influence on rats behavioral indices under the conditions of normalcy and reserpine-induced depression. On the latter model the expressivity of basic depression symptoms – hypothermia and blepharoptosis was measured.

So, in OFT in the intact animals tetrapeptide KK-1 demonstrated statistically significant (and more expressive than reference drug imipramine) sedative properties. Earlier it has been shown that KK-1 demonstrated sedative properties in other animals species, such as mice [18]. Thus, its ability to sedate CNS is non-species-specific.

Whereas reserpine inhibits CNS, decreasing locomotor, exploratory activities of mice and increasing their emotional lability in response to the distress caused by open-field space, reduction of its inhibitory action by some pharmacological agent is the marker of its antagonism with inhibitory activity of reserpine. As the result of KK-1 properties study at OFT, its ability to reduce reserpine-induced decreasing of both locomotor and exploratory activities was found. Furthermore, in OFT tetrapeptide KK-1 normalizes emotional lability of mice increased by reserpine, that indicates its stress-protective action.

Despite the absence of significant influence of tetrapeptide neuroprotector KK-1 on indices of helplessness behavior of mice in the tail suspension test

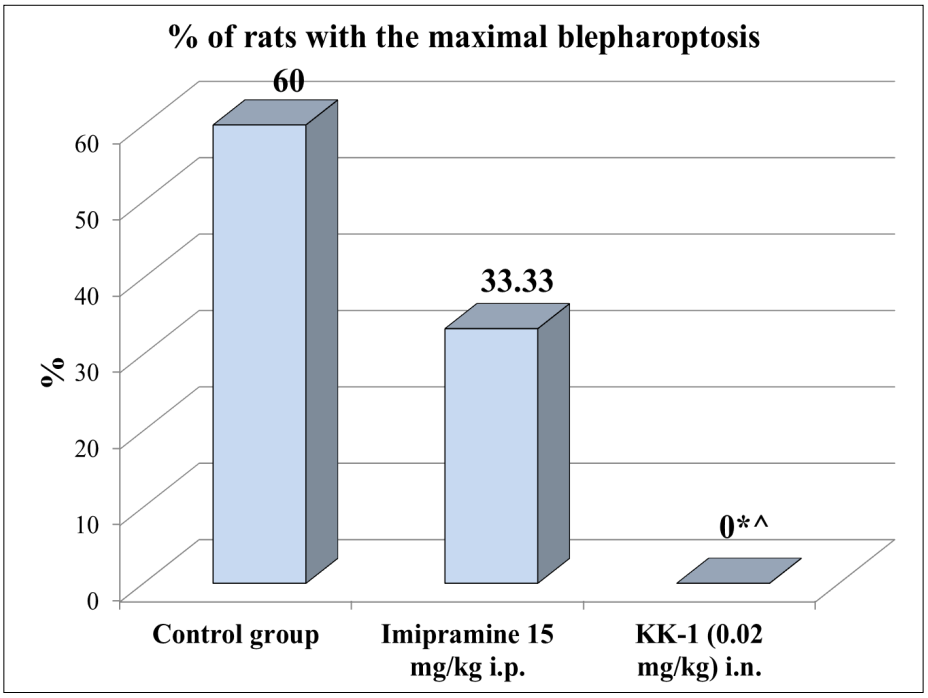

Figure 3. Influence of tetrapeptide neuroprotector KK-1 at a dose of 0.02 mg/kg i.n. and reference drug imipramine (15 mg/kg i.p.) on the number of animals with maximally pronounced blepharoptosis (reserpine-induced depression model)

Note. p≤0.05: * – compared with control group, ^ – compared with imipramine group.

[18], in the PST this tetrapeptide significantly decreased this value in rats. The investigated peptide also reduced depressivity indices of rats under the conditions of reserpine-induced depression, that was statistically significant. According to the indices in PST as follows: increase of latent time of immobilization, decrease of number of immobilizations and general time of their duration, tetrapeptide neuroprotector KK-1 exceeds activity of the classical tricyclic antidepressant imipramine. Particularly, it exceeds imipramine according to the quickness of anti-depressant action. It is well-known fact that anti-depressant effect of classic antidepressants such as tricyclic compounds (particularly imipramine) is developed gradually [22]. So, neuropeptide KK-1 is characterized not only by strongly pronounced, but also quickly developed antidepressant-like effect.

As well as imipramine, peptide KK-1 decreased the basic depression symptoms. Particularly, it reduced hypothermia level and blepharoptosis.

Thus, it has been found that new tetrapeptide neuroprotector acetyl-(D-Lys)-Lys-Arg-Arg-amide

(KK-1 demonstrates expressive antidepressant-like properties both in intact animals and in animals with reserpine-induced depression. Since depletion of brain noradrenaline pool consists at the heart of reserpine depressant action, it may be concluded that antidepressant-like activity of KK-1 may be realized through by adrenergic neurotransmitter system.

5. Conclusions.

Antidepressant-like activity of new tetrapeptide neuroprotector acetyl-(D-Lys)-Lys-Arg-Arg-amide (KK-1), homologous of $ACTH_{15-18}$ amino acids link, has been shown. It manifested both in intact animals and in animals with reserpine-induced depression.

In the intact animals in Porsolt swimming test neuroprotector KK-1 increased latent time of immobilization, decreased number of immobilizations episodes and also the general time of their duration, that was statistically significant. The same activity of KK-1 was demonstrated also in rats with reserpine-induced depression.

In the open-field test tetrapeptide neuroprotector KK-1 prevented acutely developed reserpine-induced locomotor and exploratory activity

decrease in rats. Unlike imipramine, in this test neuropeptide KK-1 decreased level of emotional reactions in rats.

Antidepressant-like action of neuroprotector KK-1 was accompanied with tendency to reserpine-induced hypothermia decreasing and statistically significant reducing of expressed blepharoptosis.

As a member of the peptidergic neuroprotectors class, KK-1 has higly pronounced psychopharmacological profile. Apparently, antidepressant-like activity of KK-1 that was shown in this study, is part of its complex neuroprotective properties. Combination of expressive neuroprotective and advantageous psychotropic properties is a valuable attribute of the modern clinically used drug. Therefore, an in-depth study of pharmacodynamic mechanisms of anti-depressant and other psychotropic activities of neuroprotective oligopeptide KK-1 is needed.

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