RESEARCH OF THE PROTECTION ACTIONS OF DERIVED 2-OXOINDOLE IN ACUTE STRESS

BADANIE OCHRONNYCH WŁAŚCIWOŚCI POCHODNYCH 2-OKSOINDOLU W OSTRYM STRESIE

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ABSTRACT

Introduction: Correction of pathological anxiety and stress level of frustration leads to the development of new anxiolytics, notably including derivatives of 2-oksoyndolyn-3-hlyoksylic acid.

The aim of work – to study the effect of 2-oksyindolin-3-hlyoksylic acid on emotional and behavioral reactions of rats subjected to behavioral stress tests of different averiability.

Materials and methods: in experiments on 150 white mature male rats Wistar investigated the effects of 2-oksoindolin (2-hydroxy-N-naphthalene-1-yl-2-(2-hydroxy-1,2-dihydro-indole-3-ylidene)-acetamide) with laboratory codes 18 when intraperitoneally administered to acute immobilization stress on Sel'ye on emotional and behavioral responses of animals to test «open field», «a raised cross maze» and test «conflict behavior» (option Vogel).

Results: established that the prophylactic use of the compound 18 in a test of "open field" warned the stress changes in the latent period of the first movement, likely increased the number of exits to the center installation, warned breach vertical and horizontal motor activity and significantly increased the number of acts of grooming and reduced the number of boluses compared with stress without correction.

Revealed changes suggest that the substance 18 prevents anxiety disorders conduct stress genesis. In the test «a raised cross maze» of 2-oksoindolin significantly increased the number of outputs rats in open arms maze and reduced the number of fecal balls compared with those in the control disorders. In the study antyconflict action found that the compound increased the number of approaches to the drinkers, but the activity in this test yielded diazepam.

Conclusion: installed conservation action anksyolityc 2-hydroxy-N-naphthalene-1-yl-2-(2-hydroxy-1,2-dihydro-indole-3-ylidene)-atsetamidu acute stress may be associated with indirect stimulation of GABA – benzdiazepin receptor complex, by strengthening endogenous GABA affinity to the receptors and/or indirect stimulation of GABA receptors by other neurotransmitter systems, including serotonergic, which makes compounds for further study the possibility of post stress with anxiety disorders.

KEY WORDS: 2-oksoindolin, acute stress, anxiety, anxiolytic effect.

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INTRODUCTION

Among the various mental disorders anxiety disorders are the most common [15]. In modern conditions of social instability and socio-economic reconstruction of society anxiety becomes a chronic phenomenon. Many stressful situations can cause anxiety and it remains even after the disappearance of the traumatic situation. Throughout life, they develop in approximately 25% of the population. When referring to doctors in General medical practice the symptoms of pathological anxiety are detected in 30-40% of patients [7]. Anxiety disorders, in addition to the General (generalized) anxiety include panic, social anxiety (phobia), agoraphobia, stress, post-traumatic and obsessive-compulsive disorder. Stress response, dissoziation (conversion), somatoform and other neurotic disorders, also belong to the group of anxiety disorders. They have a complex pathogenesis and are the result of acute anxiety. The high frequency of anxiety disorders, accession depressive component, an unfavorable course and prognosis of somatic diseases, explains the significant need for anxiolytic therapy in this group of patients [8; 17]. The proportion of patients remains resistant to the therapy or refuse that requires the development of new treatments for anxiety [13].

Psychopharmacology has been given priority of the security of treatment, focuses on the importance of matching the clinical efficacy and adverse reactions and tolerability of the drugs.

Anxiolytics in the system of prevention of stress disorders takes a well-deserved place, so neuropharmacology should be directed to the normalization of stress-realizing and stress-limiting systems of the organism, and effectively adjust streammovies of conduct disorder [1]. The derived 2-oxoindole in previous studies effectively warned somatic, metabolic disturbances, and corrected excessive lipid peroxidation in acute immobilization stress and modified the emotionally-behavioral reactions of rats in the intact animals in behavioral tests [6].

AIM OF THE STUDY

It is therefore advisable to study the effect of derived 2-oxido-3-glioxil acid on emotional and behavioral responses subjected to stress of rats in behavioral tests of various

Table I. The effect of derived 2-oxoindole-3-naftaline acid on emotionally-behavioral reactions of animals in "open field" test under acute stress (M±m).

Group of animals (n=10)	Latent period	Number of rising	Number of coming to the center	Number of crossing squares
Intact control	1,2±0,4	19,6±2,2	3,9±0,3	99,8±9,48
Control on injection (control group)	1,1±0,2	18,5±1,8	4,5±0,4	120,1±6,96
Acute stress (control pathology)	2,6±0,5*	10,6±1,1*	2,8±0,3*	51,5±3,80*
Acute stress + compound 18	1,4±0,2**	14,1±1,0**	5,6±0,4**	107±8,8**
Acute stress + diazepam	1,5±0,17	12,5±0,99	8,3±0,76**	70,1±5,67**

Notes:
1. *# – p<0.05 compared with intact control;*
2. ** – p<0.05 compared with control on injections;*
3. ** * – p<0.05 compared with the control pathology.*

aversives because of experimental anxiety has a heterogeneous character and is a companion to emotional stress [5].

MATERIALS AND METHODS

The experiments were performed on 150 white rats-males Wistar weighing 150-200 g, grown in vivarium HEE of Ukraine "Ukrainian medical stomatological Academy" (Poltava), which is equipped in accordance with existing sanitary standards. Experiments were performed in spring from 18.00 to 20.00 hours. For the study used a derivative of 2-oxoindole (2-hydroxy-N-naftaline-1-yl-2-(2-hydroxy-1,2-dihydro-indol-3-iliden)-acetamid) laboratory code 18. Substance suspendable ex tempore in water for injections, using the emulsifier "Tween-80" and was administered to animals at a dose of 12 mg per kg of body weight intraperitoneally 1 hour before the start of the playback of acute immobilization stress, which was simulated by Selye through rigid immobilization of rats on the back for three hours [2]. Animals of the control group was administered as a solvent (1 ml of water for injection with emulsifier) and subjected to the same exposure as the experimental animals. As a reference drug used diazepam ("Tarchomin S. A., Poland) at a dose of 2 mg/kg.

Emotionally-behavioral reactions of animals after stressing effects were assessed in open field test. To analyze the behavior uses the following physiological indicators: the latent period of the first move (sec.) the number of exits to the center, ambulace (horizontal activity), the number of columns (vertical activity), indicators of autonomic balance: the number of acts of grooming and acts of defecation by the number of fecal boluses [10]. Conducted research in the test elevated cross maze testing lasted 5 minutes. The study took into account the number of outputs in open sleeves, the number looking at the open sleeves and the bottom end closed and open sleeves, the time spent in the open sleeve, and the intensity of acts of defecation by the number bolus balls [10]. The influence of connections on the condition of the animals after acute stress was assessed using the test of "conflict behaviour" (option Vogel) in which he modeled the collision drinking and defensive motivation. Every attempt of taking water punished electrovalves irritation. Previously rats were subjected to water deprivation for 72 hours without limiting the consumption of dry food and develop the skill of taking water from troughs. When testing the animal was placed in a cage for 10 minutes and put electrovalve irritation (0.25 mA) whenever the taking of water from a drinking bowl. Recorded the time the latent period of the first approach to the drinking bowl (sec.) and the number of punishable attempts to quench the thirst [10].

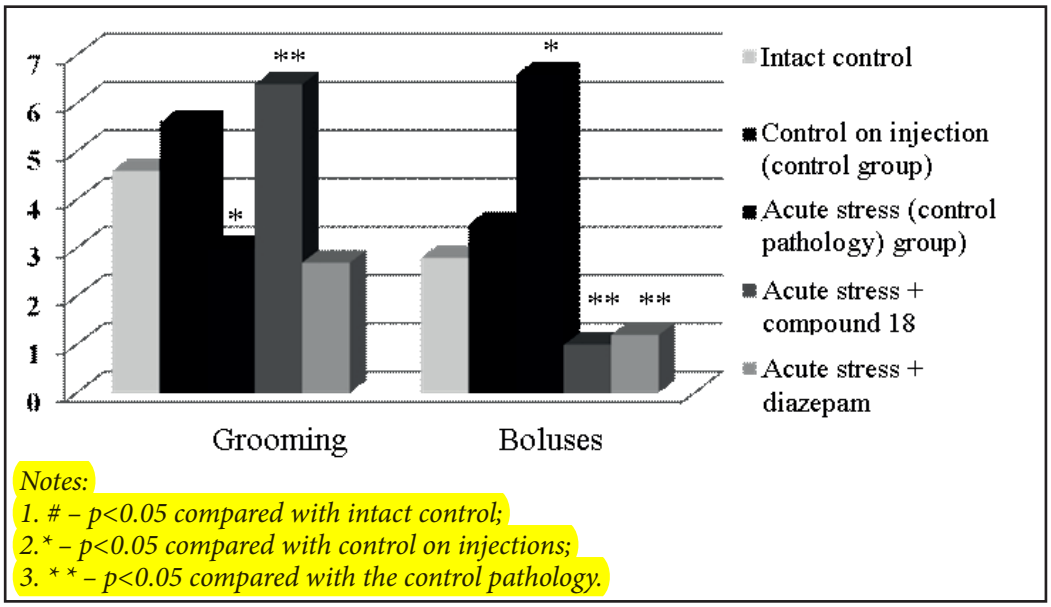
The processing of the obtained results was carried out using the programs Microsoft Statistical 6.0 using the t student criteria.

RESULTS AND DISCUSSION

After 1 h after injection of the solvent with an emulsifier in the control group significant changes of investigated parameters were noted in comparison with the values of intact animals (table. I).

After playing the acute immobilization stress in rats was observed the changes of emotional-behavioral reactions. First of all suppressed research activity, as evidenced by the increase in the latent period of the first moving 2.3 times compared to control ($p<0.01$) and a decrease in the number of outputs in the center of the "open field" in 1,6 times in comparison with indicators of intact animals, which were injected solvent ($p<0.01$). Under the influence of acute stress has varied indicators of motor activity, as indicated by the reduction of 2.3 times the number of crossed squares ($p<0.001$) and 1.9 times the number of the standing in comparison with the control group rats ($p<0,002$). In experimental pathology experimental animals, along with the violation of exploratory parameters were varied vegetative reactions, as indicated by the decrease in the number of washings 1.9 times compared to control ($p<0.0001$) and an increase in the number of boluses in 1.9 times in comparison with intact animals ($p<0.001$) (fig. 1).

Thus, after playback of acute immobilization stress decreased the activity of rats in open field test, as indicated by the increase in the latent period of the first moving and reduce the number of outputs in the centre and evidence of the violation of the processes of adaptation to new conditions of stay and the presence of feelings of anxiety and discomfort. Also raised



Notes:
1. # – p<0.05 compared with intact control;
2. – p<0.05 compared with control on injections;*
*3. * * – p<0.05 compared with the control pathology.*

Figure 1. The effect of derived 2-oxoindole on the performance of vegetative reactions of rats in "open field" test in acute immobilization stress (quantity).

research activity in the form of reducing the number of ambulance and vertical standing. The revealed changes indicate the inhibition of interest and interest in the environment. Against this background, a significant decrease in the number of acts of grooming and inversely proportional to the change in the number bolus balls are the important patterns of violations of the emotional sphere of animals, there is a sense of anxiety that accompanies exposure to stressing factor [9]. The change of emotional-behavioral reactions, obviously, testifies to the disturbing nature of stress-induced behavior disorders. These mental disorders can be regarded as a consequence of hyperproduction of hypothalamic corticalis, which is considered as a mediator of stress from anxiety, and central activation of sympatho-adrenal system, which is always observed in the effects on the body emergency factors [14].

Application of the substance 18 is reliably warned of stress change of the latent period of the first moving compared to the stress without correction. After prophylactic administration of a derivative of 2-oxoindole-3-glyoxylic acid number of outputs in a center increased 2 times as compared to that under acute stress without pharmacoproteomic ($p<0.01$) (table. 1). Motor activity was characterized by the increase in the number of crossed squares in 2.6 times ($p<0.001$) and increase in the number of the standing 1.6 times ($p<0.05$) in comparison with stress without correction. Use of a compound with the laboratory code 18 warned stress violations of motor activity of rats. A quantitative index of ambulace grew 2.1 times ($p<0.001$), significantly increased the number of vertical standing compared with stress without correction. The derived 2-oxoindole-3-glyoxylic acid stress warned of violation of the grooming that was characterized by increased frequency of washing in 2.1 times ($p<0.001$) compared with that under the action of stressing factor. It significantly reduced the number of boluses 6.6 times compared with the control pathology ($p<0.001$) (figure). In conditions of acute stress diazepam increased the number of exits in the center of the open field 3 times in comparison with stress without correction ($p<0.001$). The reference drug significantly increased the number peresta squares and reduced the number bolus balls compared with the control pathology without correction (table. 1; figure).

Compound 18 was reduced latent period of the first moving and increased the number of exits to the center "open field". So it warned the disturbed processes of adaptation to stress and reduced anxiety, which is especially enhanced under conditions of novelty under which the experimental animal is. The derived 2-oxoindole normalized reduced stress horizontal and vertical motor activity and decreased response defecation of rats. Therefore, it can be argued that the existing anxiety activity in compounds that are investigated, i.e. the ability to prevent excessive stimulation of the nervous system and optimize behavioral responses under stress.

From the above it can be stated that the substance 18 in the "open field" test prevents the development of anxiety behaviour changes stressing genesis and activity is not inferior to the reference drug diazepam.

In the next series of experiments studied the effect of compound 18 on emotional and behavioral responses of animals in the test "elevated cruciform maze". It is established that the introduction of solvent and emulsifier (tween-80) in the control group did not significantly affect physiological parameters of rats in this experimental test (table II).

Under conditions of acute immobilization stress decreased the number of outputs in open sleeves of the labyrinth by 2.4 times ($p<0.001$) compared to control (table II). Also significantly decreased the time spent and the number looking at the open sleeves compared to control injection. Stresemann contributed to the decrease in the number of looking down 1.8 times ($p<0.001$) and increased the number of bolus balls in 1.3 times in comparison with control group animals ($p<0.01$).

Thus, in the test "elevated cruciform maze" acute stress increased the phenomenon of anxiety, which was manifested by a reduction of interest in the open space in the form of reducing the amount of looking and of the sleeve and re-

Table II. The effect of derived 2-oxoindole on levels of anxiety of animals in the test "elevated cross maze" after acute immobilization stress.

Group of animals (n=10)	The number of outputs in open sleeves	The time spent in the open sleeve, sec	Number of looking into the open sleeve	Number of looking down	Number of boluses
Intact control	3,70±0,50	29,3±3,11	4,0±0,44	7,1±0,44	5,43±0,24
Control on injection (control group)	3,9±0,23	26,1±2,28	3,9±0,31	7,4±0,45	5,30±0,20
Acute stress (control pathology)	1,6±0,16 0,001	21,1±1,30 0,01	1,7±0,21 0,001	4,2±0,33 0,001	6,8±0,47 0,01
Acute stress + compound 18	2,71±0,21 0,001	24,7±1,96 -	1,8±0,25 -	4,80±0,39 -	3,1±0,31 0,001
Compound 18	5,70±0,37**	69,7±4,72**	3,90±0,31**	8,20±0,44**	2,80±0,29**
Acute stress + diazepam	2,9±0,28 0,001	28,7±2,08 0,01	2,5±0,17 0,01	5,6±0,40 0,02	1,4±0,16 0,001
Intact+diazepam	6,0+0,44**	54,1+3,51**	4,6+0,31**	7,56+0,44**	2,2+0,13**

Notes:
1. *# – p<0.05 compared with intact control;*
2. ** – p<0.05 compared with control on injections;*
3. ** * – p<0.05 compared with the control pathology.*

ducing the time spent in the open sleeve and risk assessment (reduction of looking down) and increased emotionality evidenced by the increase in the number of boluses.

Prophylactic use of a compound 18 significantly prevented decrease in the number of outputs in open sleeves of the maze and reduced the number of boluses in 2.2 times compared with that in acute stress (p<0.001) (table II). Introduction of diazepam before stresemannia rats significantly increased the number of outputs in open sleeves elevated cross maze and the time spent in it in comparison with the control pathology (table II). Also, the drug increased the number of looking into a clear sleeve 1.5 times (p<0.01) and increased the number looking down 1.3 times compared to the stress without correction (p<0.02). Against this background, the classic tranquilizer, reduced the number of boluses in 4.9 times compared with the control pathology.

Along with the study anxiaty action of compound 18 under the stress of soft to medium aversives tests used a conflict situation, a variant of Vogel.

In conditions of acute stress significantly increased the time latency of the first approach and the number of approaches to the drinker as compared with the control group animals (table III). Anticonflict the effect of derived 2-oxoindole and classic anxiaty of diazepam on the background of acute immobilization stress has undergone changes (table III). Compound 18 did not significantly affect on latent period approach to the drinker, however, increased the number of approaches to the drinking bottle 1.7 times (p<0.001) in comparison with stress without correction. Diazepam stronger warned stress-induced changes in behavior in the test of "conflict behaviour", as evidenced by a reduction of latent period to approach drinkers in 1.4 times (p<0.001) and increased the number of approaches to the drinking bottle 3.4 times (p<0.001) compared with that in the control pathology (table. III).

As you can see that anticonflict the action of connection 18 after an acute stress persisted, but was less pronounced than in the diazepam.

Therefore, under the influence of acute immobilization stress in classical tests, behavioral tests, attenuated the anxiolytic effects of compounds 18 and diazepam, which is typical for the vast majority of benzodiazepine anxiety and drugs of different structure [3; 4].

Thus stress causes changes anxiaty activity of compound 18 and diazepam in behavioral tests. Their effects depend on the degree aversives test. The effectiveness of compound 18 in the "open field" test was at the level of a reference drug, in a test of "elevated cruciform maze" decreased a little, and in the test of "conflict behaviour" there was a further decrease in the efficiency of the substance that is investigated. Thus was less effective and diazepam.

The obtained results confirm that the impact of extraordinary factors on the body suppressed the functional ability of the GABA-benzodiazepine receptor complex, and reduced volume of distribution of GABA receptors in the prefrontal cortex and other parts of the brain that regulate behavior of animals and in particular the development of anxiety [16]. During stress endogenous ligands endosan usemodule with benzoduazepine plot of the GABA receptor and disrupt binding to it exogenous ligands and diminish the signs of anxioty actions in anxiety, in particular diazepam. However, the continued effectiveness of diazepam after stress indicates a greater affinity of the drug to receptors at conservan with endogenous ligands [4]. Discovered saving anxiaty actions derived 2-oxoindole in acute stress, apparently due to the original mechanism of action, which, obviously, is to indirectly stimulate GABA-benzdiazepine receptor complex by increasing the affinity of endogenous GABA to the corresponding receptor and/or indirectly stimulate GABA receptors via other neurotransmitter systems, particularly serotonergic. Since the interrelation of these systems, particularly under pathological conditions [11].

Table III. The effect of derived 2-oxoindole on indices of animal behavior in the test of "conflict behaviour" after acute immobilization stress.

Group of animals (n=10)	The latent period of the first punishable takes water from the troughs	Number of approaches to the drinker
Intact control	134,4+10,4	81,4+7,13
Control on injection (control group)	137,4+12,2	86,7+7,56
Acute stress (control pathology)	170,2+8,69 0,05	39,4+3,26 0,001
Acute stress + compound 18	152,1+6,12	64,8+4,56 0,001
Acute stress + diazepam	120,6+9,11 0,001	133,3+5,52 0,001

Notes:
1. # – p<0.05 compared with intact control;
2. – p<0.05 compared with control on injections;*
3. * – p<0.05 compared with the control pathology.*

CONCLUSIONS

Peculiarities of anxiaty actions derived 2-oxoindole after the impact of extraordinary factors on the body involve the use of 2-hydroxy-N-naftaline-1-yl-2-(2-hydroxy-1,2-dihydro-indol-3-iliden)-acetamid when postsecretory disorders of various etiologies, complicated by anxiety.

It is planned to detect receptor and biochemical anxyolitic mechanisms of action of 2-oksoindoline in further research.

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