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PECULIARITIES OF CHANGES OF BEHAVIOURAL REACTIONS IN MATURE RATS WITH INTRANASAL INTRODUCTION OF NITROXOLINE IN VARIOUS DOSES

Abstract. *Effect of toxic substances on the human organism has been successfully reconstructed in experiments on animals. Experiments conducted on animals enable to obtain information which help to draw a conclusion between the dose of a substance and its biological effect. One of the additional sources of information is analysis of behavioural changes of animals in experiments, due to which a toxic effect of a medical agent on the nervous system can be confirmed or denied. To interpret behavioural reactions in a proper way the groups of experimental animals are formed by similar type of higher nervous activity. Nitroxoline possesses anti-tumour action and is administered in Alzheimer's disease. Special attention should be paid to the investigation of nitroxoline action on the central nervous system. Therefore, the objective of our study was to perform experimental investigation of the peculiarities and dynamics of behavioural reactions changes in albino rats in case of intranasal introduction of nitroxoline various doses as an integral index of the body physiological condition and toxic effect on the nervous system. The animals received single intranasal water solution of nitroxoline in the doses corresponding the concentrations in air 706,8 mg/m^3, 235,6 mg/m^3 and 78,5 mg/m^3. Estimate threshold of a single inhalation action of nitroxoline was 235,6 mg/m^3. The control group included rats who received an appropriate amount of distilled water. Behavioural activity of rats was examined on the first day after intoxication with various doses of nitroxoline and on the second day beginning with the experimental and control groups by means of "open field" method. Nitroxoline effect on behaviour of albino rats with intranasal introduction in the doses corresponding to the concentrations in the air 706,8 mg/m^3, 235,6 mg/m^3 and 78,5 mg/m^3 was examined. The examined levels of the drug effect were not found to produce changes of the vertical, horizontal motion activity, and hole exploratory behaviour. Changes of the parameter during two days of observation did not go beyond the limits of statistically acceptable levels and were of physiological, adaptive character. As the result of 'thiopental test' longer duration of sleep in rats after nitroxoline introduction in the dose of 706,8 mg/m^3 was detected. On the whole threshold potential is within the limits of statistically acceptable levels.*

Key words: *nitroxoline, vertical and horizontal motion activity, hole exploratory behavior, thiopental sleep.*

Introduction. Methodological diagram of examination of the drug with substantiation of a working dose in the air includes: detection of parameters of acute toxicity, detection of cumulative properties, detection of local effect on the skin and mucous membrane of the eye, detection of the parameters of subchronic toxicity, examination of remote consequences [1]. The range of pharmacological effects of doses, the mechanism of action and specific activity of the drug effect on different body systems are also considered which is indicative of a potential and real danger of the drug action.

Effect of toxic substances on the human organism has been successfully reconstructed in experiments on animals. Experiments conducted on animals enable to obtain information which help to draw a conclusion between the dose of a substance and its biological effect. One of the additional sources of information is analysis of behavioural changes of animals in experiments, due to which a toxic effect of a medical agent on the nervous system can be confirmed or denied [2].

In pharmacology and toxicology a number of behavioural tests are used based on the analysis

of spontaneous and general behavior, which in general characterize regulating, integrating, coordinating and correcting functions of the central nervous system [3].

To interpret behavioural reactions in a proper way the groups of experimental animals are formed by similar type of higher nervous activity. The animals are divided into active and passive. Active ones are characterized by clearly expressed orientation-exploring reaction to unknown surroundings, and as a result they quickly find food (conditional reflex). Passive ones are characterized by prevailing defence reaction to new surroundings, an expressed vegetative reaction is determined. To determine the type of the nervous system the following characteristics are of great importance: the time of reflex emergence, the rate of its fixation and stability, volume and duration of the reflex, the rate of its extinction and restoration, processes of excitement and inhibition [4, 5].

The method of Morris' water maze is among popular tests of behavioural reactions. The principle of this method is based on putting an experimental rat into a pool with turbid water, and a special platform is placed there. The animal is swimming and searching for the platform. The rat which found the platform is taken from the pool and in certain time it is put there to swim again but from another side. Every animal has six tries. Every next try to find the platform must be shorter and simpler. By means of this test the ability of animals to learning, cognition and remembering is determined, the dynamics of formation of general spatial memory by an animal is assessed, and the strategy of animal behavior in the course of the experiment is evaluated [5, 6].

Experiments in maze demonstrate how quickly experimental animals get information, and how effectively they use experience obtained earlier [5].

To examine social memory the ability of rats to identify the smell of their own species is used.

There are different tests to detect motion activity, coordination of movements [7], emotional reactivity [8]. These methods are: the method of «vertical» motion activity in a limited medium, open platform method, open field method, rotatory cone method and a comprehensive method with an integral assessment of behavioural parameters in animals.

The domestic literature presents data concerning changes of behavioural reactions under the effect of anthropogenic pollutants [9], stress [10], and toxic poisons: nitrobenzene [11], cadmium [12, 13], manganese [14, 15], 7-hydroxycoumarin [16], nitrate-lead intoxication [17], etc.

Nitroxoline is a synthetic uroantiseptic agent acting by the mechanism of chelation. The drug is produced by several chemical-pharmaceutical plants: joint-stock company (JSC) «Kyiv Vitamin Plant» (Kyiv), JSC «Borshchagivsky Chemical-Pharmaceutical Plant» (Kyiv), JSC «Technologist» and JSC «Vitamins» (Uman, Cherkasy region). Under conditions of technological industrial process a medical substance can penetrate into the body of workers through the respiratory tract and skin. In addition, nitroxoline possesses anti-tumour action and is indicated in case of Alzheimer disease [18]. Therefore, special attention should be paid to the study of nitroxoline effect on the central nervous system.

According to literary evidence the workers contacting with chemical substances can manifest negative signs including emotional response to external stimulants in the form of increased irritability or emotional instability, quick changes of mood [7].

Investigation of behavioural reactions in case of intranasal introduction of nitroxoline was carried out within the range of toxicological experiment concerning elaboration of hygienic regimen of an acceptable content of substances in the air of a working area at chemical-pharmaceutical enterprises.

The objective of our study was to perform experimental investigation of the peculiarities and dynamics of behavioural reactions changes in albino rats in case of intranasal introduction of nitroxoline various doses as an integral index of the body physiological condition and toxic effect on the nervous system.

Materials and methods. Investigation on laboratory animals was carried out according to methodological recommendations [4] on albino rats with the body weight 200-300g in the vivarium of Danylo Halytsky Lviv National Medical University according to sanitary-hygienic requirements. The animals were kept in cages 6

rats of the same sex in each cage, they were kept on a diet of full value, fed on at the same time and examined considering biorhythms of behavioural reactions.

The animals received single intranasal water solution of nitroxoline in the doses corresponding the concentrations in air 706,8 mg/m^3, 235,6 mg/m^3 and 78,5 mg/m^3. Estimate threshold of a single inhalation action of nitroxoline was 235,6 mg/m^3 [19]. The control group included rats who received an appropriate amount of distilled water.

Behavioural activity of rats was examined on the first day after intoxication with various doses of nitroxoline and on the second day beginning with the experimental and control groups by means of "open field" method. For this purpose the rats were placed into the manege with the size of 1 meter per meter divided into twenty-five squares in a dark room. Nine holes 5 cm in diameter were made between the squares. Every animal was placed individually in a central square of the open field. The experiment lasted for 20 minutes. The time of leaving the central square was fixed by means of a stopwatch (latent period). At the same time, the criterion of moving animals to another square was the fact of displacement of both pelvic limbs over the line dividing squares. During several minutes the number of squares where the animal entered was visually determined (horizontal motor activity), the number of raising to posterior limbs (vertical motor activity), number of "holes" smelled by a rat and looked into (hole exploratory behaviour).

Functional activity of the CNS was determined by means of thiopental test through endogastric introduction of 1% thiopental sodium solution [20]. An average duration of sleep of rats in every group was detected.

Total-threshold potential (TTP) was determined according to certain methods [21].

The results obtained were compared with the norm [22] and were statistically processed by means of Student criterion. The data are presented as a mean value and appropriate absolute error (M ± m). The difference between the examined indices was considered to be reliable with the value ($p \leq 0,05$).

While assessing toxicological basic bahevioural reactions and their certain components the vertical component of motor activity is the most sensible reflecting deep changes in the functioning of the CNS.

Results. The findings of the "open field" test on the first day showed that with an increased dose of the drug vertical activity decreases a little and approaches those of the control group indices (Table 1). On the second day of the experiment the tendency changed considerably. The parameters of the vertical activity of rats intoxicated by a maximal dose of the drug were higher than those from the control group, the lower the dose of the drug the more reliable statistical decrease of the vertical motor activity was registered. All these parameters during two days of the experiment did not exceed the borders of the acceptable levels. They were of physiological and adaptive character.

Discussion. The obtained findings of the

Table 1.

Parameters of behavioural reactions

Assessment parameters Doses	Days	Vertical motor activity (M ± m)	Horizontal motor activity (M ± m)	Hole exploratory behaviour (M ± m)	Thiopental sleep, min (M ± m)	TTP
78,5 mg/m^3	1 day	1,17 ± 0,50	3,83 ± 0,62	1,17 ± 0,35	42,0 ± 2,62	7,17±0,39
	2 day	0,80 ± 0,43	3,71 ± 1,07	1,10 ± 0,48	41,5 ± 2,86	7,5±0,97
235,6 mg/m^3	1 day	0,92 ± 0,31	5,50 ± 1,35	0,65 ± 0,44	48,16 ± 4,23	8,25±0,92
	2 day	0,90 ± 0,40	4,80 ± 0,99	1,28 ± 0,43	48,17 ± 3,89	8,00±0,75
706,8 mg/m^3	1 day	0,75 ± 0,45	4,50 ± 1,81	3,35 ± 0,97	74,33 ±2,74	7,21±0,47
	2 day	1,60 ± 0,69	5,30 ± 1,61	1,30 ± 0,45	47,67 ± 2,38	6,5±2,0
Control	1 day	0,67 ± 0,47	5,90 ± 1,35	1,42 ± 0,66	38,30 ± 2,64	11,33 ± 1,65
	2 day	1,50 ± 0,59	4,70 ± 1,42	1,80 ± 0,43	37,33 ± 1,71	11,06±1,75

*Note: *reliable difference between the parameters p<0,05.*

horizontal motor activity during 2 days varied inconsiderably concerning the control parameters, although increased parameters were registered with the dose of 235,6 mg/m^3 on the first and second days, and with the dose of 706,8 mg/m^3 on the second day, although within the borders of the accepted levels.

As the result of an open platform test with "hole exploratory behaviour" in its basis it is seen that in case of the dose lower than that of a threshold of a single inhalation action on the first and seconds days a reliable decrease of the number of squares is registered found by an animal concerning the control ones. With the dose 706,8 mg/m^3 on the first day hole exploratory behaviour was a little higher than that in the control group, but not relatively reliable ($p \leq 0,05$).

In the result of "thiopental test" the sleep of rats increased after injection of nitroxoline in the dose of 706,8 mg/m^3, reliable difference ($p<0,05$) as compared to that of the control.

Total threshold potential on the first and second days with all the doses was reduced as compared to that of the control, but this difference was not considerable ($p < 0,05$), which is indicative of adaptive processes in the body in response to a harmful factor.

Conclusions. Nitroxoline with intranasal introduction in the doses corresponding to the concentrations in the air 706,8 mg/m^3, 235,6 mg/m^3 and 78,5 mg/m does not provoke the symptoms of intoxication and neurological disorders (abnormal behavior, posture disorders, coordination disorders, muscular tone disorders etc.).

The analysis of behavioural reactions of rats being under the effect of nitroxoline demonstrated lack of changes of the vertical, horizontal motor activity, and hole exploratory behavior.

In the result of "thiopental test" an increased duration of sleep was found in rats after introduction of nitroxoline in the dose 706,8 mg/m^3.

Total threshold potential is within the borders of statistically accepted levels.

Prospects of further studies. Considering the literary evidence and our own investigation a conclusion can be suggested concerning the necessity of further studies of nitroxoline effect on the body under conditions of manufacturing the drug in order to prevent possible negative effect and occurrence of the disease among the workers of chemical-pharmaceutical industry.

References:

1. MV po obhruntuvanniu hranychno dopustymykh kontsentratsii likarskykh zasobiv u povitri robochoi zony i atmosfernomu povitri naselenykh mists. zatv. MOZ Ukrainy 21.10.2005; № 544. K, 2005. 19 p.

2. Horna OI. Zminy povedinkovykh reaktsii u tvaryn iz riznym profilem motornoi asymetrii v umovakh bolovoho stresu. Fiziol. zhurnal. 2014;60(3):34-5.

3. Melnykov AV, Kulykov MA, Novykova MR. Vыbor pokazatelei povedencheskykh testov dlia otsenky typolohycheskykh osobennostei povedenyia krыs. Zh vыssh nerv deiatelnosty. 2004;54(5):712-17.

4. Metodycheskye rekomendatsyy po yspolzovanyiu povedencheskykh reaktsyi zhyvotnыkh v toksykolohycheskykh yssledovanyiakh dlia tselei hyhyenycheskoho normyrovanyia: MR № 2166-80. Utv. MZ SSSR 12.04.1980. Kyev; 1980. 47 p.

5. Aleksandrova ZhH, Suvorov NB, Shanyn YuN, Tsыhan VN. Yndyvydualnыe osobennosty povedenyia, obuchenyia, adaptatsyy krыs k эksperymentalnыm vozdeistvyiam Fyzyolohycheskyi zhurnal SSSR ym. Y.S. Sechenova. 1984; 70(9):1294-300.

6. Golinska P. Badania na szczurach – omowienie metodologii [Electron. resource]. 2015; Access mode: http://neuropsychologia.org/badania-na-szczurach-omowienie-metodologii.

7. Vorhees Ch, Williams M. Morris water maze: procedures for assessing spatial and related forms of learning and memory [Electron. resource] 2006; – Access mode: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2895266/

8. Jamie N Justice, Christy S Carter, Hannah J Beck. Battery of behavioral tests in mice that models age-associated changes in human motor function. Age (Dordr). 2014;36(2):583-95.

9. Alline C. Animal models of anxiety disorders and stress [Electron. resource] / Rev. Bras. Psiquiatr. 2013; 35(2). – Access mode: http://dx.doi.org/10.1590/1516-4446-2013-1139.

10. Bohoiavlenska VF. Osoblyvosti formuvannia povedinkovykh reaktsii pid diieiu antropohennykh zabrudniuvachiv v eksperymenti. Suchasni problemy toksykolohii. 2011;(5):75-6.

11. Chuian EN, Hornaia OY. Moduliatsyia povedencheskykh reaktsyi krыs s raznыm profylem motornoi asymmetryy pod vlyianyem hypokynetycheskoho stressa Neirofyzyolohyia. 2010;42(3):247-53.

12. Shyrobokova LP, Khrapak VV. Dynamika zmin povedinkovykh reaktsii u bilykh shchuriv pry eksperymentalnii nitrobenzolnii intoksykatsii. Suchasni problemy toksykolohii. 2002;(1):1-6.

13. Fedorenko YuV. Sumatsiia pidporohovykh impulsiv za umov okremoi ta poiednanoi dii kadmiiu y immobilizatsiinoho stresu z urakhuvanniam typolohii povedinky eksperymentalnykh tvaryn. Medychni perspektyvy. 2015; XX(2):11-6.

14. Juostna V. Research article neurobehavioral alterations in cadmium exposed rats. International Journal of Recent Scientific Research. 2016;7(3), 9418-24.

15. Petrynych VV, Vlasyk LI. Dynamika zmin povedinkovykh reaktsii u statevozrilykh shchuriv pry marhantsevii intoksykatsii zalezhno vid shvydkosti atsetyliuvannia. Bukovynskyi medychnyi visnyk. 2010;14(2):110-2.

16. Hubar IV. Vplyv nyzkykh doz spoluk svyntsiu, rtuti, marhantsiu na povedinkovi reaktsii piddoslidnykh tvaryn zalezhno vid yikh viku. Narysy vikovoi toksykolohii. Trakhtenberh IM, editor. K: Avitsena; 2005. p.149-64.

17. Zinovieva ML, Karpezo NO, Lynchak OV. Osoblyvosti povedinkovykh reaktsii samok i samtsiv shchuriv za subkhronichnoi dii 7-hidroksykumarynu. Suchasni problemy toksykolohii. 2012;(3-4):81-5.

18. Yanchuk VV, Vlasyk LI. Dynamika zmin povedinkovykh reaktsii ta yikh struktury u infantylnykh shchuriv pry izolovanii ta kombinovanii nitratno svyntsevii intoksykatsii. Suchasni problemy toksykolohii. 2002;(4)20. Access mode: http://medved.kiev.ua/arhiv_mg/st_2002/02_4_7.htm

19. Knez D, Brus B, Coquelle N , Sosič I, Šink R, Brazzolotto X, Mravljak J, Colletier JP, Gobec S. Structure-based development of nitroxoline derivatives as potential multifunctional anti-Alzheimer agents. Bioorg Med Chem. 2015;23(15):4442-52. doi: 10.1016/j.bmc.2015.06.010.

20. Kuzminov BP, Zazuliak TS, Yaskiv HI, Turkina VA. Obhruntuvannia oriientovno bezpechnoho rivnia vplyvu nitroksolinu u povitri robochoi zony vyrobnychykh prymishchen Suchasni problemy toksykolohii, kharchovoi ta khimichnoi bezpeky. 2016;75(3):39-42.

21. Hyzhlarian MS. Nove dannыe k prymenenyiu heksenalovoi probы v toksykolohycheskom эkperymente. Hyhyena truda y profzabolevanyia 1979;(10):49-50.

22. Speranskyi SV. O preymushchestvakh yspolzovanyia narastaiushchei sylы toka pry yssledovanyy sposobnosty belakh mышei k summatsyy podporohovыkh ympulsov Farmakolohyia y toksykolohyia. 1965;(1):123-4.

23. Trakhtenberh. YM, Sova RE, Sheftel OV, Onykyenko FA. Pokazately normы u laboratornыkh zhyvotnыkh v toksykolohycheskom эksperymente (sovremennыe predstavlenyia y metodycheskye podkhodы, osnovnыe parametrы y konstantы). M: Medytsyna; 1978. 176 p.