THE STANDARDIZED MODEL OF ALCOHOLIC ANESTHESIA FOR THE PURPOSEFUL SCREENING OF ANALEPTICS

The analysis of pathogenetic mechanisms of development of urgent conditions (anesthesia, asphyxia, hypoxia, shock, collapse, bacterial intoxication, poisoning by chemical compounds or drugs suppressing the function of the central nervous system) suggests that among urgent therapies analeptic drugs (AD) are of a paramount importance. At the same time, their assortment has not only been updated in the last 50 years, but reduced to 6 drugs. This situation is conditioned by the absence of the standard for selecting AD.

Aim. To create a standard for selecting AD which can quantitatively (statistically reliable) evaluate both the level of the awakening effect in general and its mechanism (namely its analeptic action); compare and determine the priorities of further study of substances; create theoretical foundations for the purposeful search of AD and optimize the scientific research.

Materials and methods. During the research the procedure of AD selection was developed experimentally. It consists in intraperitoneal introduction of the narcotic agent (ethanol) in the optimal dose; introduction of a classical analeptic sulfocamphocaine (SCC) in the standard dose at the peak of anesthesia to one group of mice, while the second group received the substance studied (Heterocide-31) with the subsequent recording of the anesthesia duration, dynamics of the frequency of respiratory movements, assessment of the psychomotor state and physiological functions of animals during anesthesia and after awakening assuming that in the course of the experiment the specific dose-time conditions for introduction of substances are observed.

Results. The results of the study show that the maximum efficacy (18.2 %) was achieved by Heterocide-31 in the dose of 1 mg / kg, while the optimal dose of SCC (20 mg / kg) accelerated awakening of animals by 19.5 %. Thus, Heterocide-31 showed almost identical activity in the concentration of 20 times lower than SCC. The fact that after introduction of Heterocide-31 the respiratory rate (RR4) significantly increases (p <0.05) by 1.6 times already within the first minute compared to the control group, and the maximum (125) respiratory movements / min in the reference drug group is achieved only in 6 min (RR5) indicates the 6-fold advantage of Heterocide-31 by the rate of the respiratory center stimulation and allows referring the latter to a number of promising analeptics.

Conclusions. The model of pharmacological screening proposed accelerates the purposeful search of original AD, has a complete novelty, originality: easy in repeatability, economic, environmental and humanistic advantages, namely it reduces time and the number of laboratory animals, the cost of experiments, increases the information value of the experiments. The method has been tested on heterosides and suggests that derivatives of sulfur and nitrogen-containing heterocycles are promising for the search of original AD having a significant advantage over classical analeptics.

Key words: alcohol; anesthesia; heterocide; analeptic; awakening effect; respiratory rate; respiratory center

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The analysis of pathogenetic mechanisms of development of urgent conditions (anesthesia, asphyxia, hypoxia, shock, collapse, bacterial intoxication, poisoning by chemical compounds or drugs suppressing the function of the central nervous system) suggests that among urgent therapies analeptic drugs (AD) are of a paramount importance. At the same time, their assortment has not only been updated in the last 50 years, but reduced to 6 drugs (nikethamide, sulfocamphocaine, caffeine, bemegride, etimizol, corazon) with limited application [1-4].

This situation is conditioned by many reasons, namely by the absence of the generalized research standard for the AD screening model (dose-time routes for application of the substances studied and reference drugs for specific animals) [5, 6]. This makes impossible and preclude from:

- the quantitative (statistically reliable) assessment of both the level of the awakening effect in general and its mechanism (namely its analeptic action);
- comparison in the same conditions and determination of the prospects and priorities of further study of the substances;

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injected SCC in the dose of 20 mg/kg [9-11, 13, 14]. Heterocide-31 in the dose of 1.0 mg/kg. Group 3 was of anesthesia (the immobilized lateral position with intraperitoneally in the 15-th min in the third phase de-31) and the reference drug were also introduced of mice was control. The test substance (Heterocide-31) was injected 12.5 % of ethanol intraperitoneally in the dose of 5.5 mg/kg per animal [3, 4, 9-12]. Group 1 were injected AA. The reference drug was the classical compound analeptic sulfocamphocaine (SCC) stimulating the respiratory and vascular motor centers of the medulla oblongata [3, 4, 6-8].

The animals were kept under the standard conditions of the Central Research Laboratory at the National University of Pharmacy (NUP dance with the sanitary and hygiene standards: hu h) in accordance was not more than 50 %, with the temperature of 19-24 °C, and the “day-night” natural light regime, in plastic cages on a standard diet with free access to water [8]. The study was conducted according to the EU Directive 2010/10/63 EU in experiments involving animals; requirements of the “General ethical principles of experiments on animals”; the methodological recommendations of the State Pharmacological Center Ministry of Health of Ukraine on Preclinical Research of Medicines and the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1985) [7].

Mice were divided into 3 groups (n = 6). All mice were injected 12.5 % of ethanol intraperitoneally in the dose of 5.5 mg/kg per animal [3, 4, 9-12]. Group 1 of mice was control. The test substance (Heterocide-31) and the reference drug were also introduced intraperitoneally in the 15-th min in the third phase of anesthesia (the immobilized lateral position with slow breathing) [9-11]. The second group received Heterocide-31 in the dose of 1.0 mg/kg. Group 3 was injected SCC in the dose of 20 mg/kg [9-11, 13, 14].

The efficacy of all substances studied was assessed by the duration of anesthesia (DA), and the effect on RC was determined by the frequency of respiratory movements per minute (FRM/min) in different phases of anesthesia before and after the injection of Heterocide-31 or SCC. Indicators of DA and FRM / min for the first group were used as a control for other groups.

The degree of the awakening effect of Heterocide-31 and SCC was estimated by the difference in time between anesthesia (taking the position on four paws) and the time of the lateral position (LP) [9-11, 13-15]; the analeptic effect (the effect on RC) was determined according to the dynamics of FRM/min. (FRM 4-FRM 8).

FRM 1 of each animal was measured for 60 sec, starting with the moment the mice took LP. FRM 2 was counted in the 7-th min, FRM 3 – in the 14-th min of the anesthesia sleep, FRM 4 was measured (in the 15-th min) immediately after introduction of Heterocide-31 or SCC. The following values were recorded in the 20-th min (FRM 5), in the 25-th min (FRM 6) and in the 30-th min (FRM 7). After a complete awakening (position on four paws) the last measurement was carried out (FRM 8) [9-11, 13-15]; the psychomotor state of animals (disorientation or purposefulness of the movement), the level of their post-narcotic adaptation (hyperactivity or inhibition, interest in food and water), physiological reactions (hypersalivation, urination, defecation), etc., were assessed.

The reliability of the results was evaluated according to the criteria of Kruskal-Wallis and Mann Whitney using Statistica 10.0 [16].

Results and discussion

According to the results of the experiments conducted among 20 substances studied (derivatives of sulfur and nitrogen-containing heterocycles) Heterocide-31 showed a pronounced awakening effect (Tab. 1). The maximum efficiency (182 %) was achieved by Heterocide-31 in the dose of 1 mg/kg, while the optimal dose of SCC (20 mg/kg) accelerated awakening of animals by 19.5 %. Thus, Heterocrine-31 in the concentration of 20 times less than that of SCC showed almost the same activity.

The quantitative characteristics of the experiment corresponded to the behavioral ones. Animals receiving Heterocide-31 after awakening showed a clear coordination of movements (rapid targeted movement), the active use of food and water, increased diuresis and bowel movements. Mice from the SCC group after a complete awakening moved much more slowly, mostly around the perimeter of the cell, often fell, there was the lack of interest in water and food, urination was rare. Animals of the control group after ethanol anesthesia were generally disoriented and retarded for a long time (they
moved slowly, often made a lot of circular movements, there was the lack of interest in water and food, while diuresis was less pronounced).

The given observations of behavioral reactions of animals completely coincide with the traditional notions about the mechanisms of action of classical analeptic – SCC [3, 4], and ethanol [1-6, 12], which suppresses the central nervous system and causes intoxication of the organism as a whole.

Comparison of quantitative values of SCC in different phases of ethanol anesthesia with DA indicated that after introduction of ethanol, FRM 1 – FRM 4 significantly (p<0.05) decreased from 115, 107, 104, respectively, to 76 FRM/min, reaching the minimum in the control group in the 15-th min of anesthesia (Tab. 2, Fig.). After introduction of Heterocide-31 and SCC there was an immediate (at the tip of the needle) significant increase of FRM 4 in relation to the control group by 57.8 % and 38 %, respectively. Subsequent synchronous stabilization of FRM under the effect of SCC and Heterocide-31 occurred already in the 20-th min (FRM 5 – by 30.5 % and 31.5 %, FRM 6 – by 25 % and 26 %, FRM 7 by 15.8 % and 16.8 %, FRM 8 – by 5.1 % and 6.8 %, respectively) and was observed up to the total awakening of animals (FRM 8). This can be explained

| Groups                        | The average time of the lateral position | The average anesthesia duration | The awakening effect, % |
|-------------------------------|----------------------------------------|---------------------------------|------------------------|
| Ethanol (n=6)                 | 2 min. 02 sec. 115(102;125)            | 103 min. 48 sec. 6207(4208;6826) | 100 %                  |
| Ethanol + Heterocide – 31     |                                        |                                 |                        |
| 1.0 mg/kg (n=6)               |                                        | 84 min. 55 sec. 5041(4999;5241)  | 81.8 %                 |
| Ethanol + Sulfocamphocaine    |                                        | 83 min. 36 sec. 4676 (3888;5550* | 80.5 %                 |
| 20 mg/kg (n=6)                |                                        |                                 |                        |

Notes:
1) p – the level of statistical significance when comparing samples using dispersion analysis ANOVA;
2) * – the level of statistical significance when comparing samples of the groups studied with the control group using Newman-Keuls test;
3) n – the number of mice in the group.

The awakening effect of the substances studied on the model of alcoholic anesthesia

| Groups                        | The average time of the lateral position | The average anesthesia duration | The awakening effect, % |
|-------------------------------|----------------------------------------|---------------------------------|------------------------|
| Ethanol (n=6)                 | 2 min. 02 sec. 115(102;125)            | 103 min. 48 sec. 6207(4208;6826) | 100 %                  |
| Ethanol + Heterocide – 31     |                                        | 84 min. 55 sec. 5041(4999;5241)  | 81.8 %                 |
| Ethanol + Sulfocamphocaine    |                                        | 83 min. 36 sec. 4676 (3888;5550* | 80.5 %                 |
| 20 mg/kg (n=6)                |                                        |                                 |                        |

Notes:
1) p – the level of statistical significance when comparing samples using dispersion analysis ANOVA;
2) * – the level of statistical significance when comparing samples of the groups studied with the control group using Newman-Keuls test;
3) n – the number of mice in the group.

The effect of the substances studied on the frequency of respiratory movements on the model of ethanol anesthesia in mice (n = 6)

| Group | Control pathology | Ethanol + Heterocide-31 | Ethanol + Sulfocamphocaine | p    |
|-------|-------------------|-------------------------|----------------------------|------|
| FRM 1 |                   | 115 (108;120)           |                            | 0.1797 |
| FRM 2 |                   | 107 (100;118)           |                            | 0.0240 |
| FRM 3 |                   | 104 (94;110)            |                            | 0.2023 |
| FRM 4 | 76 (66;90)        | 120 (114;124)*          |                            | 0.0021 |
| FRM 5 | 95 (78;104)       | 124 (122;124)           |                            | 0.0782 |
| FRM 6 | 96 (82;110)       | 120 (116;120)*          |                            | 0.0150 |
| FRM 7 | 107 (106;110)     | 124 (118;126)*          |                            | 0.0229 |
| FRM 8 | 117 (116;132)     | 123 (114;138)           |                            | 0.7860 |

Notes:
1) p – the level of statistical significance when comparing samples using dispersion analysis ANOVA;
2) * – the level of statistical significance when comparing samples of the groups studied with the control group using Kruskal-Wallis test;
3) ** – the level of statistical significance when comparing samples with the Heterocide-31 group using Kruskal-Wallis test;
4) n – the number of mice in the group.
by the related mechanisms of exposure of both a traditional analeptic SCC and Heterocide-31 substance, which was studied for the first time, on RC [9-11, 13, 14].

The above facts of coincidence experimentally confirm the adequacy of the research model proposed and emphasize the objectivity and optimality of the chosen time of introduction of the substances studied (in the period of the maximum depth of anesthesia) [9-11, 13, 14].

The fact that after introduction of Heterocide-31 the respiratory rate (RR4) significantly increases (p <0.05) by 1.6 times already within the first minute compared to the control group, and the maximum (125) respiratory movements / min in the reference drug group is achieved only in 6 min (RR5) indicates the 6-fold advantage of Heterocide-31 by the rate of the respiratory center stimulation and allows referring the latter to a number of promising analeptics.

Thus, the method of pharmacological AD screening proposed can solve the problems of searching promising AD; it has a certain novelty, originality, economic, environmental and humanistic advantages:

- reduces the number and the cost of experiments; the number of injured and disposed laboratory animals; the time spent on the experiments;
- increases the information value of the experiments (the possibility of qualitative and quantitative statistically reliable comparison of the analeptic and side effects);
- can significantly accelerate the purposeful search for original effective analeptics and expand the critically limited range of drugs that are relevant for use in extreme life support conditions.

**CONCLUSIONS**

1. The adequacy of the method of ethanol anesthesia developed has been experimentally confirmed; it allows determining and comparing the anti-narcotic activity of promising substances and classical analeptics, as well as their effect on RC statistically reliably, objectively, qualitatively and quantitatively.

2. It has been determined that heterocides (derivatives of sulfur and nitrogen-containing heterocycles) have some advantages over classical analeptics and are a promising area for a purposeful search of AD.

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