The study of the analeptic activity of derivatives of sulfur- and nitrogen-containing heterocycles on the model of thiopental narcosis

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The range of the use of analeptics covers a number of urgent states (shock, collapse, anesthesia, asphyxia, hypoxia, bacterial intoxication with chemical compounds or drugs suppressing the CNS functions), i.e. almost all areas of urgent therapy of peacetime, military medicine and disaster medicine. However, over the past 50 years, their arsenal has not only been renewed, but even reduced to 6 drugs. This is because of the lack of standardized methods for assessing analeptic efficacy and the theoretical basis for their development.

Aim. To search and study the promising substances with the analeptic effect among derivatives of sulfur- and nitrogen-containing heterocycles on the standardized model of thiopental narcosis.

Materials and methods. The results of the experiment conducted confirm the adequacy of the method tested, which allows to compare the effect of original substances and classical analeptics on the central nervous system (CNS) and respiratory center (RC) objectively qualitatively and quantitatively (statistically reliably) by intraperitoneal administration of optimal doses: anesthesia (sodium thiopental); at the peak of anesthesia the classical analeptic sulfocamphocaine (SCC) for the first group of animals, the test substances for the second and third groups with the subsequent observation of the anesthesia sleep duration, the dynamics of the frequency of respiratory movements, the psychomotor state and the physiological functions of mice in anesthesia and after awakening.

Results. It has been found that the optimal depth and duration of anesthesia is achieved by the intraperitoneal injection of 42 mg/kg of thiopental sodium. The most effective was the traditional analeptic SCC, which in the optimal dose (20 mg/kg) accelerated the awakening of animals by 35%. Heterosides-21, -31 provided a reliable reduction in duration of anesthesia by 26.8% and 24.4% in the dose of 2 mg/kg. After introduction of the test substances the frequency of respiratory movements (RM) increased significantly (p < 0.05) for 10 min compared to the control group, reaching the maximum (85.3 DD/min) in the SCC group. It indicates its superiority in the rate of RC stimulation compared to both Heterosides, which with the concentration of 10 times less than SCC were statistically insignificantly inferior to it by 8.2% and 10.6%, respectively.

Conclusions. The model of thiopental narcosis makes it possible to statistically reliably compare the effectiveness of classical drugs and original substances by the signs of the awakening effect on the CNS and RC; it can be recommended as a reference model for the purposeful search of analeptics. On the example of studying heterosides the adequacy of the screening model proposed and the prospects of derivatives of sulfur- and nitrogen-containing heterocycles for the search of effective analeptics have been confirmed.

Key words: narcosis; sodium thiopental; analeptics; heteroside; awakening effect; respiratory center

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Дослідження аналептичної активності похідних сірко- та азотовмісних гетероциклів на моделі тіопенталового наркозу

Діапазон використання аналептиків охоплює широкий ряд невідкладних станів (шок, колапс, наркоз, аспіксія, гіпооксія, бактеріальна інтоксикація, обтурення хімічними сполуками або ліками, що пригнічують функції ЦНС), тобто майже усі сфери ургентної терапії мирного часу, військової медицини та медицини катастроф. Водночас лише за останні 50 років їх арсенал не тільки не поповнювався, а навіть скорочувався до 6 препаратів. Це зумовлено відсутністю стандартизованих методів оцінки аналептичної ефективності і теоретичних основ їх створення.

Мета дослідження – цілеспрямований пошук перспективних субстанцій з аналептичною дією серед похідних сірко- та азотовмісних гетероциклів на стандартизованій моделі тіопенталового наркозу.

Матеріали та методи. Результати проведеного експерименту підтверджують адекватність відпрацьованої методики, що дозволяє об’єктивно якісно та кількісно (статистично достовірно) порівнювати вплив оригінальних речовин і класичних аналептиків на ЦНС і дихальну систему (ДД) шляхом внутрішньоочеревинного введення оптимальних доз: наркозного засобу (тіопенталу натрію); на піку наркозу однієї групи тварин класичного аналептика сульфокамфокаїну (СКК); другій та третій – досліджуваних субстанцій (Гетерозиди-21, -31) з наступним спостереженням тривалості наркозного сну, динаміки частоти дихальних рухів, психомоторного стану та фізіологічних функцій мишей у стані наркозу та після пробудження.

Результати. Встановлено, що оптимальна глибина і тривалість наркозу досягається при внутрішньоочеревинному введенні 42 мг/кг тіопенталу натрію. Найбільш ефективним виявився традиційний аналептик СКК, який в оптимальній дозі (20 мг/кг) прискорював пробудження тварин на 35%, Гетерозиди-21, -31 забезпечували вірогідно (p<0,05) скорочення тривалості (26,8 % та 24,4 % відповідно) наркозу в дозі 2 мг/кг. Після введення досліджуваних речовин вже протягом 10 хвилин частота дихальних рухів (ДР) вірогідно (p < 0,05)
The range of the use of analeptics covers a number of urgent states (shock, collapse, anesthesia, asphyxia, hypoxia, bacterial intoxication with chemical compounds or drugs suppressing the CNS functions), i.e. almost all spheres of urgent therapy of peacetime, military medicine and disaster medicine [1].

The paradox of the current situation with analeptics is that over the past 50 years their arsenal has not only been renewed, but even reduced to 6 drugs worldwide and up to 3 in Ukraine (sulfocamphocaine, caffeine and cordiamin), which, by virtue of their peculiarities and disadvantages cannot stand modern requirements of anesthesiology and resuscitation [2].

Among the numerous references of scientific sources concerning the effectiveness of analeptics in the abovementioned cases of life support in extreme conditions there are no methodological recommendations, standardized methods for assessing their effectiveness, and even hints of the theoretical basis for their creation [1, 2].

Thus, optimization of the purposeful search of safe, analeptic agents with the wide spectrum of action is a feasible, promising and highly relevant problem, and development of standardized models of their pharmacological screening is a very important step in its solution.

The aim of the study is the purposeful search promising substances with the analeptic effect among...
derivatives of sulfur- and nitrogen-containing heterocycles on the model of thiopental narcosis (TPN) developed.

Materials and methods

The analeptic (awakening) effect of the substances was assessed in males of white nonlinear mice weighing 20-30 g. TPN (PC Kyivmedpreparat, ARTERIUM, Ukraine) was used as a suppressor of the respiratory and vascular motor centers of the brain. It is actually used in modern medicine and veterinary medicine as an anesthetic drug.

The reference drug was the classical combined analeptic sulfocamphocaine (SCC) stimulating the respiratory and vascular motor centers of the medulla oblongata [3].

The optimal doses of TPN (42 mg/kg), SCC (20 mg/kg), Heteroside-21 and -31 (2 mg/kg) were determined experimentally by titration of doses [4-6].

The mice were kept in plastic cages on a standard diet with free access to water in the conditions of the Central Research Laboratory at the National University of Pharmacy in accordance with the sanitary and hygienic norms (t = 19-24 °C, humidity not more than 50 %, “day-night” natural light regime) [7]. All studies were performed in accordance with the requirements of the “General ethical principles of experiments on animals”, the methodological recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine on Preclinical Research of Medicines, “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1985) and in accordance with EU Directive 2010/10/63 EU in experiments involving animals [8].

During the experiment the animals were divided into 4 groups (n = 6). Initially, they all were injected intraperitoneally [4-6, 9, 10]. The first group was used as a control, and mice received only TPN. The substances under study and the reference drugs were also injected intraperitoneally after the introduction of animals into the third phase of anesthesia (the immobilized side position with the slow calm breath) at the peak of anesthesia sleep (30-40 min). The second and third groups received the substances under study, namely Heteroside-21 and Heteroside-31. The fourth group was under sulfocamphocaine [11, 12].

The effectiveness of all substances under study was assessed by the duration of anesthesia (DA). The effect on the respiratory center (RC) was determined by the frequency of respiratory movements per minute (FRM/min) in different phases of anesthesia before and after the injection of awakening drugs. Indicators of DA and FRM/min in mice from group 1 were considered to be control, and the experimental groups were compared with them [11, 12].

FRM was measured for 60 sec after the TPN injection starting with the moment the mice took lateral position (LP) (FRM 1), and the following every ten minutes (FRM 1 - FRM 9, respectively). Heteroside-21, Heteroside-31 and SCC in the corresponding groups were injected at the peak of anesthesia (in 31 min) immediately after the calculation of FRM 4. The last measurement of FRM was carried out after the mice adopted the position on the four paws (complete awakening) [13]. From this moment, the psychomotor state of animals (disorientation or purposefulness of the movement), the level of their adaptation after anesthesia (restlessness, hyperactivity, interest to food and water), physiological reactions (urination, defecation) and others were assessed [9, 10, 13]. The reliability of the results obtained was evaluated according to Newman-Keuls test and Mann-Whitney test using the Statistica 10.0 software [14].

Results and discussion

The results of the study are presented in Tab. 1. In the course of the studies performed it was found that with an intraperitoneal injection of TPN in the dose of 42 mg/kg the optimal depth and duration of anesthesia achieved. Among the substances studied Heteroside-21 and Heteroside-31 showed a marked stimulant effect, which was repeatedly statistically and reliably reproduced thereafter. The maximum efficacy (26.8 %) was significantly achieved by Heteroside-21 in the dose of 2 mg/kg, while Heteroside-31 in the same dose was slightly inferior and significantly reduced the duration of anesthesia by 24.4 %. The traditional SCC analeptic, which in the optimal dose significantly accelerated the awakening of animals by 35 %, was the most effective.

Thus, both Heterosides in the concentration being 10 times less than SCC statistically insignificantly exceeded in activity only by 8.2 % and 10.6 %, respectively. It indicates that derivatives of sulfur- and nitrogen-containing heterocycles are promising for the search of original analeptics.

The quantitative characteristics of the experiment were adequate to behavioral reactions of animals. Thus, mice in the group with Heteroside-21 and Heteroside-31 right after awakening were characterized with a good movement coordination (moving by the straight line with a quick rate), active intake of water and food together with increased diuresis in the postanesthetic period. Mice from the group taking SCC after a complete awakening moved with a less rate with frequent falling down, more often around the perimeter of the cage, without particular interest to water and food, with rare urination, and further they went to hibernation (approximately for 1 hour). Animals of the control group after awakening from TPN (85 min 20 sec) were deferred and disoriented for a long period of time (stood still or moved slowly, fell down from one side to another, performed round movements, they had no interest to water and food at all) and...
after some period of time they also fell asleep for a long period of time (1-2 hours). This behavior completely coincides with the classical complex of symptoms of TPN postanesthetic intoxication [9, 10, 13].

To determine the awakening mechanism of Heteroside-21 and -31 the dynamics of the animals’ FRM change under the action of Heteroside-21 and -31 was studied in a parallel way with SCC – a classical analeptic affecting both on the respiratory and vasomotor centers of medulla oblongata.

The comparison of the results of FRM in different phases of TPN with TN (Tab. 2) showed that FRM 1– FRM 4 decreased from 78.6; 70.2; 65.3 RM/min, respectively, after the introduction of TPN, reaching the minimum of 61 RM / min (the peak of anesthesia) in the control group in 30 – 40 min of anesthesia. After the injection of Heterosides-21, -31 and SCC there was a significant increase in FRM 5 (p < 0.05) for 10 min in relation to the control group by 12.6 %, 12.5 % and 51.8 %, respectively. Then FRM indicators were divided in the following sequence for Heteroside-21, Heteroside-31 and SCC: FRM 6 significantly (p < 0.05) increased – by 33.7 %, 28.4 % and 34.2 %, respectively; FRM 7 – by 15.6 %, 17.9 %, 65 %, 63 %, 12.6 %, 12.5 %, 51.8 %, respectively.

Table 1

| Groups | The average time of the lateral position | The average anesthesia duration | The awakening effect, % |
|--------|----------------------------------------|-----------------------------|------------------------|
| TPN    | 2 min 02 sec (173.25 ±5.66)            | 85 min 20 sec (5120.2 ± 79.9) | 100 %                  |
| TPN + Heteroside–21 | 62 min 26 sec (3745.8 ± 253.3*) | 73.2 %                  |
| TPN + Heteroside–31 | 64 min 29 sec (3868.8 ± 158.5*) | 75.6 %                  |
| TPN + Sulfocamphocaine | 55 min 29 sec (3328.8 ± 224.3*) | 65 %                  |
| p      | 0.9834                                 | 0.00001                   |

Notes:
1) p – the level of statistical significance when comparing samples using ANOVA dispersion analysis;
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 substance under study on the frequency of respiratory movements of mice on the model of thiopental narcosis

Table 2

| FRM      | TPN | TPN + Heteroside–21 | TPN + Heteroside–31 | TPN + Sulfocamphocaine | p     |
|----------|-----|---------------------|---------------------|------------------------|-------|
| FRM 1    | 78.6 ± 6.2 (n=24) | 65.3 ± 6.4 (n=24) | 61.0 ± 6.0 (n=24) | 63.3 ± 1.9** (n=6) | 0.5375 |
| FRM 2    | 70.2 ± 8.3 (n=24) | 65.3 ± 6.4 (n=24) | 61.0 ± 6.0 (n=24) | 63.2 ± 2.2 **/** (n=6) | 0.2950 |
| FRM 3    | 65.3 ± 6.4 (n=24) | 61.0 ± 6.0 (n=24) | 63.3 ± 1.9** (n=6) | 85.3 ± 6.4 * (n=6) | 0.4079 |
| FRM 4    | 61.0 ± 6.0 (n=24) | 63.2 ± 2.2 **/** (n=6) | 85.3 ± 6.4 * (n=6) | 80.5 ± 4.3 * (n=6) | 0.0720 |
| FRM 5    | 56.2 ± 6.24 (n=6) | 80.5 ± 4.3 * (n=6) | 77.3 ± 6.4 * (n=6) | 80.8 ± 3.4 * (n=4) | 0.0000 |
| FRM 6    | 60.2 ± 6.0 (n=6) | 63.2 ± 2.2 **/** (n=6) | 85.3 ± 6.4 * (n=6) | 77.3 ± 6.4 * (n=6) | 0.0000 |
| FRM 7    | 66.6 ± 12.6 (n=6) | 77.0 ± 1.0 (n=3) | 78.5 ± 3.3 (n=4) | 80.5 ± 3.3 (n=4) | 0.1342 |
| FRM 8    | 67.8 ± 8.2 (n=6) | 78.0 ± 0.0 (n=1) | 78.0 ± 5.7 (n=2) | 80.8 ± 3.4 * (n=4) | 0.2631 |
| FRM 9    | 70.2 ± 10.3 (n=6) | 78.0 ± 0.0 (n=1) | 78.0 ± 5.7 (n=2) | 80.8 ± 3.4 * (n=4) | 0.0000 |

Notes:
1) p – the level of statistical significance when comparing samples using ANOVA dispersion analysis;
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 of Heteroside-21 group and Heteroside-31 group with sulfocamphocaine group using Kruskal – Wallis test.
4) n – the number of mice in the group.
the percentage equivalent for the SCC group was already absent because all mice from the group already awakened; FRM 8 was measured only for the groups of both Heterosides, and in both cases it was by 15% higher than in the control group. FRM 9 was measured only in the control group since other groups had already awakened up by that time. The results obtained coincide with the classical conception about the mechanisms of TPN action, which suppresses the CNS in general and RC in particular [1, 2, 11-13], as well as the classical analeptic – SCCM [1-3, 11, 12]. It experimentally confirms the adequacy of the study model chosen and emphasizes the objectivity and optimality of the selected time of the injection of the substances studied (during the maximum depth of anesthesia).

During the experiment an interesting fact was found that after the injection of Heteroside-21, -31 within 10 min FRM 5 significantly (p <0.05) increased compared to the control group, reaching the maximum (85.3 RM/min) in the group of SCC. It indicates an advantage of SCC by 34.7% at the rate of stimulation of RC compared to both Heterosides (Fig.). Further synchronous stabilization of FRM under the effect of the SCC and Heterosides-21, -31 occurred already in 50 min (FRM 6), it corresponded to the starting level of FRM 1 or was slightly higher, followed by a slow awakening of the animals. The comparison of indicators of FRM 7, FRM 8 and FRM 9 demonstrates that the control group (70.2 RM/min) even after complete awakening (85 min 20 sec) did not reach the starting level of FRM 1 (78.6 DR/min). This again confirms the presence of a severe post-anesthetic intoxication of TPN on the CNS in general and RC in particular (Fig.) and explains behavioral reactions (disorientation, inhibition, the lack of interest in food and water, suppression of physiological reactions).

The results of the experiment conducted confirm the adequacy of the method of thiopental narcosis developed; it allows to objectively (qualitatively and quantitatively) assess the effect of the original substances and classical analeptics on the CNS and RC.

**CONCLUSIONS**

1. The model of thiopental narcosis allows statistically significantly compare the effectiveness of classical drugs and original substances by the signs of the awakening effect on RC; it can be recommended as a reference model for the purposeful search of analeptics.

2. It has been determined that among the substances under study Heteroside-21 and Heteroside-31 are practically not inferior in efficiency to sulfocamphocaine, and have greater advantage in dose.

3. Derivatives of sulfur- and nitrogen-containing heterocycles are promising for the search of original analeptics.

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