UDC 616.853:633.881::547.918:615.213:615.217.3:612.82 (215)

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To cite this article: Tsyvunin V., Shtrygol' S., Lytkin D., Taran A., Shtrygol' D. (2025). Rol endohennoho dyhitalisopodibnoho ta neirotrofichnykh faktoriv u protysudomnii dii dyhoksynu ta valproatu [Role of endogenous digitalis-like and neurotrophic factors in the anticonvulsant effect of digoxin and valproate]. *Fitoterapiia. Chasopys – Phytotherapy. Journal,* 3, 210–220, doi: https://doi.org/10.32782/2522-9680-2025-3-210

ROLE OF ENDOGENOUS DIGITALIS-LIKE AND NEUROTROPHIC FACTORS IN THE ANTICONVULSANT EFFECT OF DIGOXIN AND VALPROATE

Actuality. *The addition of nonclassical antiepileptic drugs, such as the cardiac glycoside digoxin, to the treatment regimens of refractory epilepsy may be one of the effective ways to control the disease. However, the mechanism of its anticonvulsant action, in particular the effect on the cerebral neurotrophin pathway, as well as endogenous digitalis-like factor, remains incompletely understood.*

Thus, the aim of the study was to elucidate the impact of digoxin, sodium valproate and their combination on the cerebral levels of endogenous digitalis-like factor (EDLF), as well as neurotropic factors – nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) – under conditions of experimental chronic epileptogenesis in aspect of interhemispheric asymmetry.

Material and methods. *The model of chronic epileptogenesis, kindling induced pentylenetetrazole (PTZ) in mice, has been used. Sodium valproate (150 mg/kg intragastrically) and digoxin (0.8 mg/kg subcutaneously) – both per se and in combination – were administered 30 min before PTZ (30 mg/kg intraperitoneally).*

After 16 days, EDLF, NGF and BDNF have been identified in the whole brain as well as in right and left hemispheres separately.

Research results. *It was confimed that the combination of digoxin with sodium valproate significantly more clearly prevents the development of seizures than monotherapy. It was established that EDLF does not significantly participate in either the development of seizures or the anticonvulsant effect of sodium valproate and digoxin. It was determined that sodium valproate, digoxin, and their combination have a pronounced neuroprotective effect by restoring the content of BDNF (but not NGF) in the brain. It was proven that the interhemispheric asymmetry of EDLF, as well as NGF and BDNF is not only a sign of experimental epileptogenesis, but also*

a feature of the central effect of sodium valproate and digoxin. Correlation analysis indicates a complex interaction between EDLF and neurotrophic factors.

Conclusion. *The obtained results expand the understanding of the mechanisms of anticonvulsant action of digoxin and sodium valproate and may be important in the development of new therapeutic approaches for epilepsy, in particular its refractory forms.*

Key words: *pentylenetetrazole-induced kindling, digoxin, valproate, endogenous digitalis-like factor, nerve growth factor, brain-derived neurotrophic factor, hemispheres.*

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Бібліографічний опис статті: Цивунін В., Штриголь С., Литкін Д., Таран А., Штриголь Д. (2025). Роль ендогенного дигіталісоподібного та нейротрофічних факторів у протисудомній дії дигоксину та вальпроату. *Фітотерапія. Часопис,* 3, 210–220, doi: https://doi.org/10.32782/2522-9680-2025-3-210

РОЛЬ ЕНДОГЕННОГО ДИГІТАЛІСОПОДІБНОГО ТА НЕЙРОТРОФІЧНИХ ФАКТОРІВ У ПРОТИСУДОМНІЙ ДІЇ ДИГОКСИНУ ТА ВАЛЬПРОАТУ

Актуальність. *Додавання некласичних протисудомних препаратів, зокрема серцевого глікозиду дигоксину, до схем лікування рефрактерної епілепсії може бути одним з ефективних способів контролю захворювання. Однак механізм його протисудомної дії, зокрема вплив на церебральні нейротрофіни, а також ендогенний дигіталісоподібний фактор, залишається не до кінця вивченим.*

Мета дослідження *– з'ясування впливу дигоксину, вальпроату натрію та їх комбінації на рівень ендогенного дигіталісоподібного фактору (EDLF), фактору росту нервів (NGF) та мозкового нейротрофічного фактору (BDNF) в умовах експериментального хронічного епілептогенезу в аспекті міжпівкульової асиметрії.*

Матеріал і методи. *Використано модель хронічного епілептогенезу – кіндлінгу, індукованого пентилентетразолом (ПТЗ), у мишей. Вальпроат натрію (150 мг/кг внутрішньошлунково) та дигоксин (0,8 мг/кг підшкірно) – як окремо, так і в комбінації – уводили за 30 хвилин до ПТЗ (30 мг/кг внутрішньочеревно). За 16 днів визначали вміст EDLF, NGF та BDNF у всьому мозку, а також у правій і лівій півкулях окремо.*

Результати дослідження. *Підтверджено, що комбінація дигоксину з вальпроатом натрію значно виразніше запобігає розвитку судом, ніж монотерапія окремими препаратами. Встановлено, що EDLF не бере суттєвої участі ані в розвитку*

судом, ані у протисудомній дії вальпроату натрію та дигоксину. Визначено, що вальпроат натрію, дигоксин та їх комбінація мають виражений нейропротекторний ефект, відновлюючи уміст BDNF (але не NGF) у мозку. Доведено, що міжпівкульна асиметрія EDLF, а також NGF та BDNF є не лише ознакою експериментального епілептогенезу, але й особливістю центрального ефекту вальпроату натрію та дигоксину. Кореляційний аналіз вказує на складну взаємодію між EDLF та нейротрофічними факторами.

***Висновок.** Отримані результати розширюють розуміння механізмів протисудомної дії дигоксину та вальпроату натрію, та можуть бути важливими в розробці нових підходів до лікування епілепсії, зокрема її рефрактерних форм.*

***Ключові слова:** пентилентетразоловий кіндлінг, дигоксин, вальпроат, ендогенний дигіталісоподібний фактор, фактор росту нервів, мозковий нейротрофічний фактор, півкулі мозку.*

Introduction. Actuality. Despite the wide range of classical antiepileptic drugs (AEDs) with different mechanisms of action, as well as the active introduction into clinical practice of innovative drugs with unique (often multiple) molecular targets of influence on epileptogenesis, such as brivaracetam, perampanel, retigabine, stiripentol, the problem of pharmacotherapy of refractory epilepsy still remains extremely relevant (Elkommos, 2022). One of the promising concepts, which has been confirmed both in experimental conditions and in the clinic, involves prescribing to patients with resistant seizures, in addition to first-line AEDs, adjuvant drugs that are not classical anticonvulsants, but are able to modulate their effectiveness (Tsyvunin, 2020). The cardiac glycoside digoxin, a substance of plant origin, is the most studied such medicine. There is considerable evidence that digoxin at low (subcardiotonic) doses is an effective adjuvant to many classical AEDs in seizure models with different neurobiochemical pathogenesis (Tsyvunin, 2022). In the pentylenetetrazole-induced kindling model in mice, it has been established that the leading mechanisms of the anticonvulsant action of digoxin are the effects on GABAergic inhibition in the brain (Tsyvunin, 2023a) as well as on neuroinflammation (Tsyvunin, 2023b). However, other ways of realizing this effect of cardiac glycoside cannot be excluded. The role of neurotrophic factors in the mechanisms of anticonvulsant action is well known. Epilepsy, like experimental seizure models, is characterized by deprivation of cerebral and plasma levels of the main neurotrophins – nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), while classical AEDs usually contribute to the restoration of their expression, which is associated with an improvement in the clinical course of the disease (Iughetti, 2018; Min, 2021).

Endogenous digitalis-like factor (EDLF) is an endogenous inhibitor of Na^+/K^+-ATPase and is involved in the regulation of electrolyte balance, membrane potential, and neuronal excitability. Its level increases in response to stressful, ischemic, and inflammatory stimuli. Despite numerous references to EDLF in the context of cardiovascular pathology, its role in the development or inhibition of neuronal hyperexcitability in epilepsy has been almost completely unexplored, which, given its ability to maintain intra- and extraneuronal balance of Na^+ and K^+ ions, requires appropriate clarification (Buckalew, 2015). Given that digoxin is an exogenous analog of EDLF, the study of this pathway in epileptogenesis as well as anticonvulsant activity realization is of particular relevance.

The relationship between EDLF and neurotrophic factors in the context of the anticonvulsant effect of drugs has also not been studied, in particular in the context of neurochemical asymmetry between the cerebral hemispheres, which may play a significant role in seizure activity (Kavraiskyi, 2017). These aspects fully relate to one of the most prescribes AED, sodium valproate, the anticonvulsant effect of which is potentiated by digoxin (Tsyvunin, 2022).

Therefore, the ability of digoxin and valproate to affect the cerebral neurotrophic link and endogenous digitalis-like factor as possible targets of their own anticonvulsant potential – both when used separately and in combination – needs to be clarified.

The aim of the study is to elucidate the impact of digoxin, sodium valproate and their combination on the cerebral levels of endogenous digitalis-like factor, as well as neurotropic factors – nerve growth factor and brain-derived neurotrophic factor – under conditions of experimental chronic epileptogenesis in aspect of interhemispheric asymmetry.

Materials and research methods. This work is a part of the scientific project "Rationale for improving the treatment of multidrug-resistant epilepsy through the combined use of classical anticonvulsant medicines with other drugs" (№ 0120U102460, 2020/2022) supported by the Ministry of Health of Ukraine and carried out at the expense of the State Budget of Ukraine.

The study was conducted on 40 outbred male albino mice weighing 20–24 g obtained from the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy (Kharkiv, Ukraine). Animals were kept in a well-ventilated room on a standard vivarium diet (laboratory rodent chow) with free access to water under controlled conditions (20–24 °C and 50% humidity with a 12 h light/dark cycle).

Experiment was carried out in accordance with the principles and requirements of the EU Directive 2010/63/EU (2010) on the protection of animals used for

scientific purposes and approved by the local Bioethical Committee (Protocol № 3, September 10, 2020).

Pentylenetetrazole-induced kindling

Murine model of chronic epileptogenesis, kindling induced pentylenetetrazole (PTZ), has been chosen (Tsyvunin, 2023a; Tsyvunin, 2023b).

For the experiment all animals were randomly divided into 5 groups 8 mice in each: 1 – vehicle control (VC); 2 – positive control (PC), PTZ-induced kindling without treatment; 3 – PTZ-induced kindling + sodium valproate; 4 – PTZ-induced kindling + digoxin; 5 – PTZ-induced kindling + sodium valproate + digoxin.

For kindling modeling, PTZ was used in a dose of 30 mg/kg intraperitoneally for 16 days. All studied medicines were administered 30 min before PTZ. Control mice (groups 1 and 2 – vehicle control and positive control, respectively) received intragastrically and subcutaneously solvent (water) in a volume of 0,1 ml/10 g body weight for 16 days. Digoxin was used subcutaneously at a subcardiotonic dose of 0,8 mg/kg ($^1/_{10}$ LD_{50}), which showed an anticonvulsant effect previously. Sodium valproate was administered intragastrically at a dose of 150 mg/kg (½ ED_{50}) (Tsyvunin, 2023a; Tsyvunin, 2023b).

Medicines have been used in the form of officinal drugs: digoxin (Digoxin-Zdorovie® 0,25 mg/ml solution for injection, DNCLZ / Zdorovie, Ukraine) and sodium valproate (Depakine®, 57,64 mg/ml syrup, Sanofi Aventis, France). Pentylenetetrazole (PTZ) has been used in the form of a substance (Sigma, USA).

The latency of the first seizures in the groups, as well as the number of days with and without convulsions were determined.

Collection and preparation of biomaterial

After 16 days of PTZ-induced kindling, animals were euthanized by dislocation of the cervical vertebrae under light chloroform anesthesia, their brains were removed, divided into hemispheres and shockly frozen with liquid nitrogen. Biological samples stored in a freezer at –70°C. Before examining the hemispheres were homogenized with standard phosphate-buffered saline (buffer-to-material ratio is 10:1) at a tempetature of −4 °C and thrice centrifugated at 6 000 × g for 10 min.

Enzyme immunoassay

EDLF, NGF and BDNF have been identified in homogenates of right and left hemispheres separately as well as in the combined homogenate, which corresponded to the whole brain.

All studies conducted by enzyme-linked immunosorbent assay (ELISA) using mouse species-specific kits has been performed in full accordance with the manufacturer's instructions. Detection has been performed on the LabAnalyt M201 Microplate Reader (Granum).

MyBioSource ELISA kits (USA): Endogenous Digitalis-Like Factor, ELISA Kit (MBS3809501), Nerve Growth Factor (NGF), ELISA Kit (MBS263575), Brain-derived neurotrophic factor (BDNF), ELISA Kit (MBS355435) have been used.

Statistical analysis

Statistical processing of the results was performed using the licensed program Statistica 10.0 (StatSoftInc., serial number STA999K347156-W).

The results were expressed as mean ± standard error of the mean (M±m) as well as median and lower and upper quartiles (Me [Q_{25} – Q_{75}]). Statistical differences between groups were analyzed using a parametric Student t-test and a non-parametric Mann – Whitney U test. Spearman's correlation coefficient (ρ) was used to establish the relationship between indicators. The levels of statistical significance were considered as $p < 0,05$ and 0,01.

Research results and their discussion. The features of the course of kindling, in particular, the effectiveness of the impact of valproate, digoxin and their combination on chronic epileptogenesis, are given in table 1. It was established that the administration of subthreshold doses of PTZ is characterized by the appearance of seizures after 4 days of the experiment, then – daily from 5 to 16 days – clinically pronounced clonic-tonic paroxysms are observed in animals of the PC group. Valproate in a subeffective dose slightly increases the latent period of the appearance of the first seizures – up to 6 days, and also provides reliable protection of animals from convulsions – up to 9 days. Digoxin *per se* had a comparable effect on the course of kindling with valproate, prolonging the latent period of seizures to 7 days, and also increasing the number of days without convulsions in the group to 10 ($p < 0,05$) relative to the PC. Meanwhile, the combination of valproate with digoxin provided a statistically significant protective effect, completely preventing the development of convulsions in mice in the group throughout the 16 days of the experiment.

The results of determining the content of EDLF in the whole mice brain as well as its different hemispheres under the influence of digoxin and sodium valproate in the model of PTZ-induced kindling are given in table 2. It was found that in the VC group there was no interhemispheric asymmetry of the cerebral content of EDLF; at the same time, a statistically significant negative correlation between the hemispheres of the brain was revealed.

PTZ-induced kindling under these conditions was not associated with changes in the cerebral EDLF level in the brain as a whole and in the left hemisphere. At the same time, in the right brain hemisphere of mice from the PC group, a certain decrease in this indicator was observed compared with VC, but this difference did not

Table 1

The course of pentylenetetrazole-induced kindling in mice under the influence of valproate, digoxin and their combination

Group	Latency, days	Days	
		without convulsions	With convulsions
Positive control, PTZ (n = 8)	4	1–4 (4)	5–16 (12)
Valproate (n = 8)	6	1–6, 8, 10, 12 (9*)	7, 9, 11, 13–16 (7*)
Digoxin (n = 8)	7	1–7, 9–11 (10*)	8, 12–16 (6*)
Valproate + Digoxin (n = 8)	16	1–16 (16**$^{\#\#\wedge\wedge}$)	(0**$^{\#\#\wedge\wedge}$)

Statistically significant differences:
* – $p < 0,05$; ** – $p < 0,01$ – compared with positive control, PTZ;
$^{\#\#}$ – $p < 0,01$ – compared with valproate;
$^{\wedge\wedge}$ – $p < 0,01$ – compared with digoxin.

Table 2

Impact of valproate, digoxin and their combination on the endogenous digitalis-like factor content in the brain of mice under the pentylenetetrazole-induced kindling, M ± m; Me [Q_{25} – Q_{75}]

Group		EDLF, ng/g			Spearman's correlation between hemispheres, ρ
		whole brain	right hemisphere	left hemisphere	
Vehicle control (n = 8)		31,86 ± 1,42 32,77 [28,44; 34,44]	32,88 ± 2,41 33,29 [29,39; 37,67]	30,84 ± 1,58 32,45 [27,65; 33,84]	**−0,74** **p < 0,05**
Pentylenetetrazole-induced kindling	Positive control (n = 8)	30,76 ± 2,35 34,16 [21,85; 37,15]	26,86 ± 4,00 21,85 [19,03; 39,03]	34,67 ± 1,79 34,94 [33,69; 36,85]	−0,14 p > 0,05
	Valproate (n = 8)	27,60 ± 1,72 25,81 [24,20; 30,77]	24,66 ± 2,14$^{\#}$ 24,20 [19,6; 28,23]	30,46 ± 2,38 27,79 [25,53; 33,97]	+0,55 p > 0,05
	Digoxin (n = 8)	27,96 ± 2,23 26,86 [22,98; 35,44]	28,74 ± 2,93 26,04 [25,16; 35,04]	27,18 ± 3,54 29,42 [18,54; 35,44]	+0,60 p > 0,05
	Valproate + Digoxin (n = 8)	27,75 ± 2,36 28,68 [21,45; 32,16]	27,02 ± 2,91 27,04 [21,45; 32,16]	28,48 ± 3,90 28,68 [20,81; 36,54]	−0,52 p > 0,05

EDLF – endogenous digitalis-like factor.
Statistically significant differences:
$^{\#}$ – $p < 0,05$ – compared with vehicle control (within one hemisphere).

reach statistical significance. The correlation relationship, although it remains negative, is significantly inferior in strength to the coefficient of the group of healthy animals.

Sodium valproate also caused a reduction in EDLF level compared with VC group, reaching the statistical significance ($p<0.05$) in the right hemisphere. The correlation between the hemispheres changes its direction and acquires a moderately positive character.

The effect of digoxin, as well as the combination of digoxin with sodium valproate on the cerebral EDLF level was similar to the effect of classical AED *per se*. However, both in hemispheres separately and in the whole brain, the reduction of the indicator in relation to VC had the character of an improbable trend, not reaching the level of statistical significance. The only difference was the change in the correlation between the hemispheres, which became negative in the combination group and almost reached the level of VC.

Table 3 shows the results of the study of the effect of digoxin and sodium valproate both in monotherapy and in combination on the cerebral content of NGF. As with EDLF, the absence of differences in the expression of this neurotrophin in the cerebral hemispheres was established, although the correlation between the hemispheres here is positive.

PTZ-induced kindling predictably caused a significant decrease in the NGF compared with VC: more than

Table 3

Impact of valproate, digoxin and their combination on the nerve growth factor content in the brain of mice under the pentylenetetrazole-induced kindling, M ± m; Me [Q_{25} – Q_{75}]

Group		NGF, pg/g			Spearman's correlation between hemispheres, ρ
		whole brain	right hemisphere	left hemisphere	
Vehicle control (n = 8)		4 248,81 ± 298,83 4 297 [3 432; 5 101]	4 260,38 ± 448,32 4 076,5 [3 432; 5 109,5]	4 237,25 ± 426,24 4 596 [3 365,5; 5 101]	+0,57 p > 0,05
Pentylenetetrazole-induced kindling	Positive control (n = 8)	2 228,72 ± 263,10$^{\#\#}$ 1 933 [1 360; 3 154,5]	2 985,75 ± 333,78***** 3 154,5 [2 142; 3 692,5]	1 471,69 ± 146,64$^{\#\#}$ 1 360 [1 155,5; 1 791,5]	+0,10 p > 0,05
	Valproate (n = 8)	2 620,44 ± 234,07$^{\#\#}$ 2 705,5 [1 892; 3 131]	3 090,0 ± 356,23 3 085 [2 665; 3 363,5]	2 150,88 ± 211,92$^{\#\#\S}$ 1 933 [1 771,5; 2 444]	−0,13 p > 0,05
	Digoxin (n = 8)	1 389,06 ± 160,04$^{\#\#\S\wedge\wedge}$ 1 417,5 [934,9; 1 995]	1 251,99 ± 245,47 $^{\#\#\S\wedge\wedge}$ 1 417,5 [578,4; 1 832]	1 526,14 ± 210,11$^{\#\#}$ 1 417,5 [1 247,5; 2 120]	−0,11 p > 0,05
	Valproate + Digoxin (n = 8)	1 475,79 ± 260,58$^{\#\#\S\wedge\wedge}$ 1 230 [804,8; 1 771]	1 367,48 ± 489,25 $^{\#\#\S\wedge}$ 804,75 [624,85; 1 429]	1 584,10 ± 219,73$^{\#\#\wedge}$ 1 398,5 [1 230; 1 791,5]	+0,72 p < 0,05

NGF – nerve growth factor.
Statistically significant differences:
***** – p < 0,05 – right to left hemisphere within one study group;
$^{\#\#}$ – p < 0,01 – compared with vehicle control (within one hemisphere);
§ – p < 0,05; §§ – p < 0,01 – compared with positive control (within one hemisphere);
$^{\wedge}$ – p < 0,05; $^{\wedge\wedge}$ – p < 0,01– compared with valproate (within one hemisphere).

twice in the whole brain and three times in the left hemisphere (p < 0,01). It is in this group of animals that a significant interhemispheric asymmetry of the neurotrophin content is observed: the decrease in NGF levels in the right hemisphere relative to healthy animals has a tendency and is significantly more than twice as high as in the left hemisphere (p < 0,05). At the same time, the correlation between the hemispheres is practically absent.

Interhemispheric asymmetry was also verified in the sodium valproate group. Unlike PC, against the background of the influence of this AED there was no significant difference between the NGF content in different hemispheres, only in the whole brain and the left hemisphere there is a statistically significant depletion of the NGF compared with the vehicle control – by 1,6 and 1,9 times (p < 0,01), respectively. The decrease in the neurotrophin content in the right hemisphere was only a tendency. There is a certain restoration of the NGF level in the left hemisphere, since there is a statistically significant difference with a similar indicator in the left hemisphere of animals in the PC group – almost by 1,5 times (p < 0,05). The weak correlation between the hemispheres in this case becomes negative.

Digoxin during PTZ-induced kindling causes a significant reduction in cerebral NGF content without interhemispheric asymmetry – probably not only compared with VC, but also with positive control and sodium valproate: in the whole brain – by 3,1, 1,6 and 1,9 times, respectively, in the right hemisphere – by 3,4, 2,4 and 2,5 times, respectively. Although the left hemisphere ratio does not differ from PC, there is a statistically significant decrease compared with VC – by 2,8 times (p < 0,01), as well as an unreliable trend reduction compared with sodium valproate – by 1,4 times (p > 0,05). The weak correlation between the hemispheres is negative, as in the classical AED group.

The combination of sodium valproate with digoxin has almost the same effect on cerebral NGF content as digoxin monotherapy. In the whole brain and the right hemisphere, a statistically significant reduction in the level of neurotrophin is observed compared with VC, PC and sodium valproate *per se*: by 2,9, 1,5, 1,8 and by 3,1, 2,2, 2,3 times, respectively. At the same time, the indicator of the left hemisphere does not differ from PC and digoxin *per se*, demonstrating statistical significance only compared with healthy animals and classical AED – by 2,7 and 1,4 times, respectively. Interestingly, the correlation between the hemispheres, as in the case of EDLF, acquires a significant (p < 0,05) positive character, close in value to the coefficient of the VC group.

The results of the study of the effect of sodium valproate, digoxin and their combination on the content of

BDNF in the brain during PTZ-induced kindling are given in table 4. It was found that the level of neurotrophin in healthy animals slightly prevails in the right hemisphere of the brain – almost by 1,3 times, but this difference is not statistically significant. As in the case of NGF, the correlation between the content of BDNF in different hemispheres is moderately positive.

PTZ-induced kindling caused a pronounced depletion of the BDNF level in the brain, statistically significant with respect to VC in the whole brain and the right hemisphere – by 2,1 and 2,8 times, respectively, as well as a pronounced, but not significant, tendency to decrease this indicator in the left hemisphere by 1,6 times ($p > 0,05$). The interhemispheric correlation coefficient did not change and remained at the level of healthy mice.

Sodium valproate, digoxin and their combination demonstrate a pronounced restoration of cerebral BDNF content to the level of healthy animals. A statistically significant increase in the pool of this neurotrophin in all three groups compared with PC is observed in the whole brain ($p < 0,01$) and in the right hemisphere ($p < 0,05$). The increase in the indicator in the left hemisphere, although quite pronounced, does not reach the level of statistical significance. Thus, valproate increases BDNF expression compared with PC by 1,9, 2,7 and 1,4 times for the whole brain, right and left hemispheres, respectively. Digoxin has an almost identical effect, restoring BDNF content compared with untreated animals: by 2,0 times in the whole brain, 2,7 times in the right and 1,5 times in the left hemispheres. The correlation of BDNF content between hemispheres in animals receiving both valproate and digoxin *per se* is positive, stronger and statistically significant ($p < 0,05$) in the classical AED group.

Combined use of valproate with digoxin normalizes cerebral BDNF content, increasing the level of neurotrophin compared with PC in the whole brain, as well as in the right and left hemispheres separately – by 1,9, 2,2 and 1,8 times, respectively. Interhemispheric correlation is almost absent: Spearman's coefficient becomes very weakly negative, approaching zero.

The correlation matrix between the content of EDLF, NGF and BDNF in the whole brain and individual hemispheres is given in table 5. In the VC group, a distinct statistically significant negative relationship was established between NGF and BDNF in the right hemisphere. PTZ-induced kindling is associated with a strong reliable positive relationship between EDLF and NGF in the left hemisphere. A significant positive correlation ($p < 0,05$) was verified between EDLF and BDNF in the left hemisphere on the background of sodium valproate. For digoxin, in turn, a significant negative correlation was found between EDLF and BDNF in the whole brain.

The obtained results indicate the absence of a significant role of EDLF itself both in the development of experimental seizures and in the implementation of the central effects of sodium valproate and digoxin.

The pronounced reduction in cerebral NGF and BDNF content against the background of chronic administration of PTZ, which proves the neurodegenerative effects of kindling, is apparently associated with

Table 4

Impact of valproate, digoxin and their combination on the brain-derived neurotrophic factor content in the brain of mice under the pentylenetetrazole-induced kindling, M ± m; Me [Q_{25} – Q_{75}]

Group		BDNF, pg/g			Spearman's correlation between hemispheres, ρ
		whole brain	right hemisphere	left hemisphere	
Vehicle control (n = 8)		1 229,38 ± 102,91 1 230,5 [921,4; 1 498,5]	1 371,23 ± 157,46 1 343,5 [953,5; 1 682]	1 087,53 ± 121,84 1 216 [798,2; 1 302,5]	+0,55 p > 0,05
Pentylenetetrazole-induced kindling	Positive control (n = 8)	584,32 ± 127,26 ## 477,9 [95,5; 1 049,5]	485,48 ± 172,23 # 359,25 [95,5; 766,7]	683,17 ± 192,22 677,15 [157,5; 1 095,5]	+0,55 p > 0,05
	Valproate (n = 8)	1 139,93 ± 131,52§§ 1 034 [871,5; 1 259,5]	1 294,00 ± 224,44§ 1 065 [920,9; 1 585]	985,85 ± 130,27 1 034 [706,2; 1 172]	**+0,74** **p < 0,05**
	Digoxin (n = 8)	1 168,97 ± 135,63§§ 1 016,9 [750,2; 1 709,5]	1 290,19 ± 219,28§ 1 412 [797,7; 1 825]	1 047,75 ± 162,97 921,35 [750,2; 1 284,9]	+0,55 p > 0,05
	Valproate + Digoxin (n = 8)	1 139,58 ± 127,15§§ 1 110,5 [750,2; 1 443]	1 053,18 ± 162,92§ 985,9 [674,6; 1 443]	1 225,99 ± 201,52 1 201,5 [883,2; 1 477,5]	–0,07 p > 0,05

BDNF – brain-derived neurotrophic factor.
Statistically significant differences:
– $p < 0,05$; ## – $p < 0,01$ – compared with vehicle control (within one hemisphere);
§ – $p < 0,05$; §§ – $p < 0.01$ – compared with positive control (within one hemisphere).

Table 5

Spearman's correlations between EDLF, NGF and BDNF in the whole brain and separate hemispheres, *ρ*

Pairs of indicators	Vehicle control	Pentylenetetrazole-induced kindling			
		Positive control	Valproate	Digoxin	Valproate + Digoxin
Whole brain					
EDLF – NGF	+0,24	−0,11	−0,18	−0,13	+0,02
EDLF – BDNF	+0,30	+0,38	+0,38	**−0,55***	−0,02
NGF – BDNF	−0,48	−0,24	+0,37	+0,29	+0,26
Right hemisphere					
EDLF – NGF	+0,17	−0,02	+0,04	−0,43	+0,05
EDLF – BDNF	+0,35	+0,47	+0,48	−0,57	+0,69
NGF – BDNF	**−0,87***	−0,43	+0,13	+0,29	+0,24
Left hemisphere					
EDLF – NGF	+0,29	**+0,83***	+0,01	+0,04	+0,32
EDLF – BDNF	+0,18	+0,11	**+0,72***	−0,51	−0,48
NGF – BDNF	−0,65	+0,19	+0,46	+0,23	+0,44

* – statistically significant correlation ($p < 0,05$).

neuronal hyperexcitability, neuroinflammation and oxidative stress, which lead to dysregulation of growth factors and a decrease in their expression (de Souza, 2019; Kazmi, 2020; Kılıç, 2025; Tekgul, 2020; Tirassa, 2007). Although valproate and digoxin do not have a significant normalizing effect on the content of NGF (valproate only tends to increase the content of this neurotrophin, while digoxin and the combination cause a statistically significant reduction in it compared with PC), both drugs in monotherapy and in combination restore the level of BDNF, which indicates their pronounced neuroprotective effect.

The role of NGF in epileptogenesis is ambiguous. On the one hand, its decrease is associated with neurodegenerative processes. On the other hand, NGF is able to change the balance between the inhibitory (GABAergic) and excitatory (glutamatergic) systems, and high levels of this neurotrophin can promote the sprouting of mossy fibers in the hippocampus, which is associated with the development of chronic epilepsy (Vast'ianov, 2012). This necessitates caution in interpreting changes in its content under the influence of a convulsant and the studied medicines. Thus, the decrease in NGF content observed against the background of sodium valproate, digoxin, and their combination may be associated with a specific effect on the signaling pathways of neuronal growth and requires additional in-depth study.

The effect of sodium valproate on the neurotrophic link of epileptogenesis may be associated with both a direct effect on the expression of neurotrophin genes through histone acetylation (Green, 2017), and with indirect antioxidant properties and the ability to modulate apoptosis and neuroinflammatory processes (Harrison, 2015; Terzioğlu Bebitoğlu, 2020). Digoxin, which has a proven effect on the activity of cerebral Na^+,K^+-ATPase (Tsyvunin, 2023a), may affect the level of neurotrophins through Ca^{2+}-dependent mechanisms, and changes in the correlations between EDLF, NGF and BDNF may be associated with the impact of the cardiac glycoside on the electrolyte balance of neurons (Buckalew, 2015).

In general, the immunohistochemical results, especially the established pronounced positive impact of the medicines on the content of BDNF in the brain, correspond to the clinically proven anticonvulsant effect of valproate and digoxin *per se*, as well as their combination. Therefore, the restoration of the cerebral BDNF can be considered a key marker of the anticonvulsant effect.

Although the combination of medicines showed a more pronounced clinical effect than the individual components *per se*, preventing the development of spontaneous convulsions, it did not have a synergistic effect on the neurotrophin link in the pathogenesis of seizures, possibly due to different interactions of sodium valproate and digoxin at the level of cellular signaling.

Thus, the restoration of BDNF levels after treatment can be considered as a marker of neuroprotection and, possibly, indirectly, of anticonvulsant action. NGF demonstrates complex dynamics: although its decrease indicates neurodegeneration, at the same time, overexpression of NGF can contribute to the formation of a hyperexcitable network by inducing mossy fiber sprouting in the hippocampus (Vast'ianov, 2012). Thus, the decrease in NGF during therapy may be adaptive in nature. Such a complex role of NGF in the pathogenesis of epilepsy clearly requires further research.

Correlation analysis of EDLF, NGF and BDNF contents in the whole brain, as well as in the right and left hemispheres separately indicates that the changes in

these factors after the administration of the medicines are complex, and individual statistically significant relationships may indicate sophisticated, apparently compensatory interactions in neurochemical systems.

The role of interhemispheric neurochemical asymmetry in epileptogenesis, as well as in the mechanisms of the anticonvulsant effect of individual AEDs, has been repeatedly discussed previously for GABA, glutamate, glutamate decarboxylase, serotonin, nitric oxide, and NO synthase (Emelianova 2008; Iarosh, 2014; Kavraiskyi, 2017). Although no neurochemical asymmetry of EDLF and NGF content between the cerebral hemispheres is observed in healthy animals, the average level of BDNF is 26% higher in the right hemisphere – tendentiously. Obvious interhemispheric asymmetry is detected in the PC group for all studied parameters: a more pronounced decrease in EDLF and BDNF is observed in the right hemisphere, NGF – in the left (statistically significant). Sodium valproate in the model of chronic epileptogenesis causes a significant falling in EDLF and increases the content of BDNF exclusively in the right hemisphere, and also enhances the production of NGF only in the left hemisphere. Digoxin affects EDLF and NGF in both hemispheres, but more significantly increases the BDNF in the right one. A combination of both medicines has an interhemispheric effect similar to digoxin. Correlation analysis additionally confirms the profound differences in the impact of medicines on the content of EDLF and neurotrophins in different hemispheres of the brain – by changing not only the strength, but also the direction of the relationship.

Thus, the interhemispheric neurochemical asymmetry of EDLF, as well as NGF and BDNF, can be considered not only a proven sign of experimental epileptogenesis, but also one of the features of the implementation of the anticonvulsant effect of sodium valproate and digoxin.

Conclusions. The anticonvulsant efficacy of digoxin, sodium valproate, and their combination has been studied in a model of chronic epileptogenesis (pentylenetetrazole-induced kindling), as well as the role of endogenous digitalis-like factor (EDLF), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF) in the mechanisms of action of these medicines in view of interhemispheric asymmetry.

1. In the pentylenetetrazole-induced kindling model in mice, it was shown that the combination of digoxin (0.8 mg/kg) with sodium valproate at a dose of 150 mg/kg (½ ED_{50}) significantly more clearly prevents the development of seizures than digoxin and valproate *per se*.

2. It was established that EDLF does not significantly participate in either the development of seizures or the anticonvulsant effect of sodium valproate and digoxin.

3. It was determined that sodium valproate, digoxin, and their combination have a pronounced neuroprotective effect by restoring the content of BDNF (but not NGF) in the brain during pentylenetetrazole-induced kindling.

4. It was proven that the interhemispheric asymmetry of EDLF, as well as NGF and BDNF is not only a sign of experimental epileptogenesis, but also a feature of the central effect of sodium valproate and digoxin.

5. Correlation analysis indicates a complex interaction between endogenous digitalis-like and neurotrophic factors, which may be important in the development of new therapeutic approaches for epilepsy, in particular its refractory forms.

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Стаття надійшла до редакції 27.01.2025
Стаття прийнята до друку 30.06.2025
Опубліковано: 15.10.2025

Conflicts of interest. The authors have no conflict of interest to declare. The authors alone are responsible for the content and writing of this article.

Authors' contribution:
Tsyvunin V.V. – collection and analysis of literature, experimental data collection, statistical analysis of data, conclusions, article writing;
Shtrygol' S.Yu. – idea, concept and design of the study, correction of the article;
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