Recommended by Doctor of Medicine, professor S. M. Drogovoz

UDC 615.217:615.213:547.853:001.891.5 DOI: 10.24959/cphj.17.1412

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The effect of 1-(4-metoxyphenyl)-5-{2-[4-(4-metoxyphenyl) piperazine-1-yl]-2-oxoethyl}-1,5-dihydro-4H-pyrazole[3,4-D] pyrydine-4-one and sodium valproate on the level of inhibitory and excitatory neurotransmitters in the brain in the hemispheric asymmetry aspect

Epilepsy affects about 1 % of the world population. Recently, epileptology considers the cerebral hemispheres (CH) as separate components, which differ due to the neurochemical hemisphere asymmetry.

Aim. To study the level of neurotransmitters and their correlation in the CH of mice receiving 1-(4-metoxyphenyl)-5-{2-[4-(4-metoxyphenyl) piperazine-1-yl]-2-oxoethyl}-1,5-dihydro-4H-pyrazole[3,4-D]pyrydine-4-one (compound 78553) and sodium valproate in intact animals, and the pentylenetetrazol kindling model.

Materials and methods. The brains of 44 mice used were divided into separate hemispheres and frozen. The levels of serotonin, GABA, glutamate, aspartate were determined by high voltage electrophoresis, and glycine – by thin-layer chromatography. Compound 78553 was administered in the dose of 200 mg/kg, valproate sodium – 300 mg/kg.

Results. The hemispheric asymmetry of the level of neuroactive amino acids is absent in intact mice, and the serotonin level is higher in the left hemisphere (LH). Compound 78553 increases the levels of glycine, aspartate, serotonin without affecting GABA and glutamate. Sodium valproate elevates the GABA level and lowers the levels of other mediators. PTZ kindling causes a marked imbalance of neurotransmitters in the CH, increases the levels of excitatory neurotransmitters, reduces the inhibitory amino acids and depletes serotonin in the LH and the right hemisphere (RH). Compound 78553 in PTZ kindling increases the levels of GABA and glycine in the CH, restores their physiological level, raises the serotonin level and reduces the amount of excitatory amino acids up to the intact group level without the balance restoration between the hemispheres. Sodium valproate increases the level of GABA more efficiently than compound 78553, but has a weaker effect on glycine, restores the normal level of glutamate, slightly reducing the aspartate level. A moderate recovery effect is observed for serotonin in the RH, while in the LH it is almost absent. The hemispheric symmetry is recovered for glycine and aspartate.

Conclusions. Correlation analysis indicates that there is the conjugation deviation of the neurotransmitter exchange for acids and serotonin between the brain hemispheres in the PTZ kindling model. The changes in direction and the strength of relationships between the neurotransmitter levels indicate the differences between biochemical mechanisms of the anticonvulsant action of compound 78553 and sodium valproate. The strong effect of sodium valproate on GABA and glutamate in epilepsy can cause its high efficiency. A stronger effect on the metabolism of serotonin and glycine can partially explain the antiepileptic effect of compound 78553.

Key words: [3,4-D]pyrydine-4-one derivatives; anticonvulsants; hemispherical asymmetry; inhibitory and excitatory neurotransmitters

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Вплив 1-(4-метоксифеніл)-5-{2-[4-(4-метоксифеніл)піперазин-1-іл]-2-оксоетил}-1,5-дигідро-4Н-піразоло[3,4-D]піридин-4-ону та вальпроату натрію на вміст гальмівних та збуджувальних нейротрансмітерів у головному мозку в аспекті міжпівкульової асиметрії

На епілепсію страждає близько 1 % населення світу. Останнім часом епілептологія розглядає півкулі головного мозку (ГМ) як нерівнозначні складові, які відрізняються за рахунок нейрохімічної асиметрії.

Мета роботи – дослідити вміст нейромедіаторів у півкулях головного мозку мишей та кореляційні зв'язки між ними на тлі прийому 1-(4-метоксифеніл)-5-{2-[4-(4-метоксифеніл)піперазин-1-іл]-2-оксоетил}-1,5-дигідро-4Н-піразоло[3,4-D]піридин-4-ону (сполука 78553) та вальпроату натрію в інтактних тварин та на моделі пентилентетразолового (PTZ) кіндлінгу.

Матеріали та методи. Використано головний мозок 44 мишей, який розтинали на півкулі і заморожували. Серотонін, ГАМК, глутамат, аспартат визначали методом високовольтного електрофорезу, гліцин – методом тонкошарової хроматографії. Сполуку 78553 вводили у дозі 200 мг/кг, вальпроат натрію – 300 мг/кг.

Результати. В інтактних мишей міжпівкульова асиметрія вмісту нейроактивних амінокислот відсутня, а рівень серотоніну вище у лівій півкулі (ЛП). Сполука 785533 збільшує вміст гліцину, серотоніну і аспартату без

впливу на ГАМК і глутамат. Вальпроат натрію збільшує рівень ГАМК, інших медіаторів – зменшує. PTZ кіндлінг викликає виразний дисбаланс медіаторів у ГМ, збільшує вміст збуджувальних нейротрансмітерів, зменшує рівень гальмівних амінокислот та виснажує запаси серотоніну як у ЛП, так і в правій півкулі (ПП). Сполука 78553 за PTZ кіндлінгу збільшує вміст ГАМК і гліцину в ГМ, відновлює їх фізіологічну симетрію в півкулях, підвищує рівень серотоніну та зменшує кількість збуджувальних нейроамінокислот майже до рівня інтактного контролю без відновлення їх балансу між півкулями. Вальпроат натрію ефективніше за сполуку 78553 збільшує рівень ГАМК, проте слабше впливає на гліцин, відновлює нормальний рівень глутамату, слабко впливаючи на вміст аспартату. Помірний відновлювальний ефект спостерігається для серотоніну в ПП, а в ЛП він майже відсутній; міжпівкульова симетрія відновлюється для гліцину та аспартату.

Висновки. Кореляційний аналіз свідчить про порушення спряженості обміну нейротрансмітерних амінокислот і серотоніну в півкулях головного мозку під впливом PTZ, а зміни напрямку і сили зв'язків між вмістом нейротрасмітерів вказують на відмінності біохімічних механізмів протисудомної дії сполуки 78553 та вальпроату натрію. Саме потужна дія останього на ГАМК і глутамат при епілепсії може зумовлювати його високу ефективність. Протиепілептичний ефект сполуки 78553 частково можна пояснити сильнішим впливом на обмін серотоніну та гліцину.

Ключові слова: похідні піразоло[3,4-D]піридин-4-ону; протисудомні засоби; міжпівкульова асиметрія; збуджувальні та гальмівні нейротрансмітери

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Влияние 1-(4-метоксифенил)-5-{2-[4-(4-метоксифенил) пиперазин-1-ил]-2-оксоэтил}-1,5-дигидро-4Н-пиразол [3,4-D]пиридин-4-она и вальпроата натрия на содержание тормозных и возбуждающих нейротрансмиттеров в головном мозге в аспекте межполушарной асимметрии

Эпилепсией страдает около 1 % населения мира. В последнее время эпилептология рассматривает полушария головного мозга (ГМ) как неравнозначные составляющие, которые отличаются за счет нейрохимической асимметрии.

Цель работы – исследовать содержание нейромедиаторов в полушариях ГМ мышей и корреляционные связи между ними на фоне приема 1-(4-метоксифенил)-5-{2-[4-(4-метоксифенил)пиперазин-1-ил]-2-оксоэтил}-1,5-дигидро-4Н-пиразол [3,4-D]пиридин-4-она (соединение 78553) и вальпроата натрия у интактных животных и на модели пентилентетразолового (PTZ) киндлинга.

Материалы и методы. Использован мозг 44 мышей, который разделяли на полушария и замораживали. Серотонин, ГАМК, глутамат, аспартат определяли методом высоковольтного электрофореза, глицин – методом тонкослойной хроматографии. Соединение 78553 вводили в дозе 200 мг/кг, вальпроат натрия – 300 мг/кг.

Результаты и их обсуждение. У интактных мышей межполушарная асимметрия содержания нейроактивных аминокислот отсутствует, а уровень серотонина выше в левом полушарии (ЛП). Соединение 78553 увеличивает содержание глицина, серотонина и аспартата без влияния на ГАМК и глутамат. Вальпроат натрия увеличивает уровень ГАМК, других медиаторов – уменьшает. PTZ киндлинг вызывает выраженный дисбаланс медиаторов в ГМ, увеличивает содержание возбуждающих нейротрансмиттеров, уменьшает уровень тормозных аминокислот, истощая при этом запасы серотонина как в ЛП, так и в правом полушарии (ПП). Соединение 78553 за PTZ киндлинга увеличивает содержание ГАМК и глицина в ГМ, восстанавливает их физиологическую симметрию в полушариях, повышает уровень серотонина и уменьшает количество возбуждающих нейроаминокислот почти до уровня интактного контроля без восстановления их баланса между полушариями. Вальпроат натрия эффективнее соединения 78553 увеличивает уровень ГАМК, слабее влияет на глицин, восстанавливает нормальный уровень глутамата, слабо влияя на содержание аспартата. Умеренный восстанавливающий эффект наблюдается для серотонина в ПП, а в ЛП он почти отсутствует. Межполушарная симметрия восстанавливается для глицина и аспартата.

Выводы. Корреляционный анализ свидетельствует о нарушении сопряженности обмена нейротрансмиттерных аминокислот и серотонина в головном мозге под влиянием PTZ, а изменения направления и силы связей между содержанием нейротрансмиттеров указывают на различия биохимических механизмов противосудорожного действия соединения 78553 и вальпроата натрия. Именно мощное действие последнего на ГАМК и глутамат при эпилепсии может обусловливать его высокую эффективность. Противоэпилептический эффект соединения 78553 частично можно объяснить более сильным влиянием на обмен серотонина и глицина.

Ключевые слова: производные пиразол[3,4-D]пиридин-4-она; противосудорожные средства; межполушарная асимметрия; возбуждающие и тормозные нейротрансмиттеры

The pathogenesis of epilepsy (E) is based on excessive neuronal discharges due to pathology of cell membranes and imbalance of excitatory and inhibitory neurotransmitters of the brain. The pathological activity of the brain has epileptic signs of individual neurons and neuronal systems in general [1]. Despite the widespread E the neurochemical nature of convulsive states has not been studied [2].

In this aspect, the problem of the brain morphological and neurochemical asymmetry draws attention. Many researches confirm the unequal activity

of the left (LH) and the right hemisphere (RH) of the human B [3, 4, 5]. This asymmetry is important in the normal brain activities and affects the clinical symptoms in E [1]. The focal process and variety of clinical manifestations make E a convenient model for studying the asymmetry of the brain hemispheres. It is important to improve the diagnosis and treatment of E. Most studies concern the permanent and paroxysmal psychopathological activity in E depending on localization of the epileptic focus activity.

It has been found that the LH can generate focal seizures more frequently than the RH in patients with schizophrenia and E [6]. The functional hemispheric asymmetry can modulate the severity of depressive symptoms, be manifested by different characteristics of focal lateralization in E [7] and greater vulnerability of the LH to epileptogenesis [4]. However, there are no verified data of the relationship of the functional sensorimotor asymmetry and specific psychopathology. The imbalance of inhibitory (GABA, glycine) and excitatory (glutamate, aspartate) neurotransmitters plays an important role in the convulsive status [8]. There are open questions regarding the changes in the balance of neurotransmitter amino acids during pharmacotherapy of E. There is a need to clarify the role of serotonin in epileptogenesis. Depletion of serotonin reserves in the brain is registered in E [9]. It is unknown how serotonin is involved in development of seizures, how its level changes under the effect of antiepileptic drugs (AEDs), and how it correlates with the level of excitatory and inhibitory amino acids considering the hemisphere asymmetry.

Pyrimidine derivatives attract attention because of the opportunity to introduce various substituents that exhibit a wide range of activities, including the anticonvulsant one. The original pyrazolo [3,4-D] pyridine-4-one derivative – 1-(4-metoxyphenyl)-5-{2-[4-(4-metoxyphenyl)piperazine-1-yl]-2-oxoethyl}-1,5-dihydro-4h-pyrazole[3,4-d] pyrydine-4-one (the laboratory code 78553) was synthesized at the Department of Pharmaceutical Chemistry in the National University of Pharmacy under the supervision of professor V. A. Georgiyants. It revealed strong anticonvulsive properties in models with different pathogenesis, the polytropic mechanism of action and the favorable psychotropic effect [10, 11]. The impact of compound 78553 on the balance of transmitters in the brain remains unknown.

The aim of this study was to study the level of inhibitory and excitatory amino acids and serotonin in the cerebral hemispheres of mice and the correlation between them in the context of the hemisphere asymmetry in intact animals and in the pentylenetetrazole-induced (PTZ) kindling model when receiving 1-(4-metoxyphenyl)-5-{2-[4-(4-metoxyphenyl) piperazine-1-yl]-2-oxoethyl}-1,5-dihydro-4H-pyrazole[3,4-d] pyrydine-4-one and sodium valproate.

Materials and Methods

The studies were conducted in 44 albino mature male mice weighing 18-25 g. Animals were kept in the vivarium of the Central Research Laboratory at the National Pharmaceutical University under the standard conditions with the daylight duration of 12-13 hours in compliance with the sanitary and hygienic norms and the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The experiments were conducted according to the General Ethical Principles and Guidelines for Experiments on Animals.

Animals were randomly divided into 6 groups: (1) intact control (n = 10); (2) intact mice (n = 8) receiving compound 78553 as a suspension in Tween-80 in a single dose of 200 mg/kg [10]; (3) intact animals (n = 7) receiving the reference drug sodium valproate ("Depakin", batch 472, Sanofi-Aventis, France) in a single dose of 300 mg/kg [10]. Mice of other three groups were subjected to a 15-day kindling [12]. Each day mice received PTZ ("Sigma", USA) injection (i.p.) in an aqueous solution in the sub-threshold dose of 30 mg/kg: (4) the group of control pathology (n = 8) and the group (5, 6) of mice receiving compound 78553 (n = 6) and the reference drug (n = 5) as described above. Compound 78553 and sodium valproate were introduced intragastrically through a probe for 30 min before killing mice of groups 2, 3 and prior each PTZ injection for other groups (4–6). After mice decapitation the brain was rapidly removed, weighed, cut on hemispheres and frozen with liquid nitrogen, then stored for analysis at -40^{0}C.

The levels of GABA, aspartic and glutamic acid were determined by high voltage electrophoresis [13]. The separation was carried out in the pyridine-acetate buffer at a voltage of 600v for 3 hours. The frozen brain was powdered. Extraction was performed in 96 % alcohol in a boiling water bath for 19 min; the ratio of alcohol–tissue was 10 : 1. The glycine level was determined by thin layer chromatography on Silufol plates [14] in the *n*-butanol – glacial acetic acid – water solvent system in the ratio of 90:10:25. The standard sample of glycine (Sigma, USA) was used.

The results obtained were processed statistically using STATISTICA 11.0 for Windows. Accuracy of intergroup variances was assessed using Student's t-test under normal distribution and nonparametric Mann–Whitney U test when samples were not normally distributed [19]. The relationship between the levels of neurotransmitters was analyzed by Pearson correlation coefficient. Differences were considered to be significant at $p < 0.05$.

Table 1

The effect of compound 78553 and sodium valproate on the levels of neurotransmitters in the separate brain hemispheres in intact mice after a single injection (n = 25)

Group	Right hemisphere		Left hemisphere		Significant differences of LH-RH
	Value	The change in relation to IC, %	Value	The change in relation to IC, %	
GABA, mcmol/g					
IC	2.85±0.09		2.91±0.05		
Compound 78553	3.09±0.06	+8.4	2.91±0.11	0	
Sodium valproate	4.03±0.04* #	+41.5	3.81±0.10* #	+31.2	p<0.05
Glycine, mcmol/g					
IC	6.44±0.18		6.11±0.13		
Compound 78553	9.95±0.13*	+54.5	10.75±0.20*	+75.9	p<0.01
Sodium valproate	3.78±0.16* #	–41.3	3.93±0.15 #	–35.7	
Glutamate, mcmol/g					
IC	9.94±0.12		10.08±0.12		
Compound 78553	10.38±0.17	+4.4	9.93±0.17	–1.4	
Sodium valproate	8.17±0.08* #	–17.9	9.12±0.10* #	–9.5	p<0.01
Aspartate, mcmol/g					
IC	4.42±0.21		4.54±0.20		
Compound 78553	5.16±0.07*	+16.9	5.44±0.13*	+19.8	
Sodium valproate	4.31±0.09#	–2.3	4.05±0.06#	–10.8	
Serotonin, nmol/g					
IC	145.96±2.70		168.81±3.03		p<0.01
Compound 78553	168.01±1.31*	+15.1	181.04±0.59*	+7.2	p<0.01
Sodium valproate	140.24±0.58 #	–3.9	154.61±1.65*#	–8.4	p<0.01

Note. IC – intact control, statistically significant differences: * – in relation to intact control (p<0.01), # – in relation to compound 78553 (p<0.01).

Results and Discussion

The amount of inhibitory and excitatory amino acids in the RH and the LH of intact animals is significantly equal (Tab. 1); however, there is a slight tendency to dominance in the LH (except glycine). This confirms the results [15, 16]. As for the level of serotonin there is the asymmetry between the cerebral hemispheres. The homogenate of the LH contains significantly more serotonin on average by 15.7 % than the RH, which is typical for mice [17]. The higher level of excitatory neurotransmitters in the LH can explain more frequent epileptic activity in this hemisphere [4].

In intact animals compound 78553 does not affect the balance of GABA-glutamate, but equally in both hemispheres increases the aspartate level by 16.9-19.8 % and strongly increases the level of glycine, mostly in the LH (at 75.9 %). The glycinergic action is confirmed by a moderate anticonvulsant activity on the model of strychnine-induced seizures [10]. The level of serotonin statistically significantly increases in both hemispheres with the prevalence in the RH, the hemisphere asymmetry retains, and it explains the moderate antidepressant properties of compound 78553 to some extent [11].

In intact mice sodium valproate affects the exchange of amino acids mainly in the RH (except aspartate). This result dissociates with the results on rats, which mainly affects the LP [16]. Probably, the difference is explained by specific species, different dose and the use of a solvent. The reference drug significantly increases the level of GABA in both hemispheres by 31.2-41.5 % and reduces the level of excitatory amino acids – glutamate by 9.5-17.9 % ($p < 0.01$) and aspartate by 2.3-10.8 % ($p > 0.05$). Unlike compound 78553, sodium valproate significantly reduces the level of glycine in the brain by 35.7-41.3 % and serotonin – by 8.4 % in the LH. Sodium valproate has a stronger effect on GABA and glutamate ($p < 0.01$), while compound 78553 – on aspartate, glycine and serotonin ($p < 0.01$).

The results of the study on the kindling model are given in Tab. 2. PTZ equally decreases the level of both inhibitory neurotransmitters (by approximately 70 %), strongly increases the level of glutamate, especially in the LH (by 84.4 %) and has less effect on the level of aspartate. The increase of the aspartate level occurs in the RH (+ 62.4 %).

Table 2

The effect of pentylentetrazole, compound 78553 and sodium valproate on the levels of neurotransmitters in the separate brain hemispheres in mice on the model of chronic epileptogenesis (kindling) (n = 29)

Group	Right hemisphere			Left hemisphere			Significant differences of LH-RH
	Value	The change in relation to:		Value	The change in relation to:		
		IC, %	PTZ, %		IC, %	PTZ, %	
GABA, mcmol/g							
IC	2.85±0.09			2.91±0.05			
PTZ	0.76±0.01*	–73.4		0.95±0.02*	–67.3		p<0.01
PTZ +compound 78553	1.77±0.04* ^	–38.1	+133.2	1.65±0.04*^	–43.2	+73.9	
PTZ + Sodium valproate	2.14±0.02*^#	–24.8	+183.0	2.35±0.02*^#	–19.3	+146.9	p<0.01
Glycine, mcmol/g							
IC	6.44±0.18			6.11±0.13			
PTZ	2.07±0.07*	–67.9		1.79±0.08*	–70.7		p<0.05
PTZ + compound 78553	4.19±0.12*^	–34.9	+102.4	4.37±0.18* ^	–28.5	+144.1	
PTZ + Sodium valproate	2.84±0.09*^#	–55.9	+37.2	3.07±0.10*^ #	–49.8	+71.5	
Glutamate, mcmol/g							
IC	9.94±0.12			10.08±0.12			
PTZ	17.30±0.11*	+74.0		18.58±0.13*	+84.4		p<0.01
PTZ + compound 78553	11.17±0.13*^	+12.4	–35.4	10.04±0.06^	–0.3	–45.9	p<0.01
PTZ + Sodium valproate	9.46±0.11**^#	–4.9	–45.3	10.62±0.04*^#	+5.4	–42.8	p<0.01
Aspartate, mcmol/g							
IC	4.42±0.21			4.54±0.20			
PTZ	7.17±0.05*	+62.4		6.73±0.08*	+48.2		p<0.01
PTZ + compound 78553	4.21±0.03^	–4.7	–41.3	4.53±0.09^	–0.3	–32.8	p<0.05
PTZ + Sodium valproate	6.19±0.09*^#	+40.2	–13.7	6.11±0.04*^ #	+34.5	–9.3	
Serotonin, nmol/g							
IC	145.96±2.70			168.81±3.03			p<0.01
PTZ	41.75±0.74*	–71.4		61.18±0.57*	–63.8		p<0.01
PTZ + compound 78553	101.50±0.86*^	–30.5	+143.1	133.47±1.96*^	–20.9	+118.2	p<0.01
PTZ + Sodium valproate	61.54±0.74*^#	–57.8	+47.4	68.66±0.74*^#	–59.3	+12.2	p<0.01

Note. IC – intact control, statistically significant differences: * – in relation to intact control (p<0.01), ** – in relation to intact control (p<0.05), ^ – in relation to PTZ (p<0.01), # – in relation to compound 78553 (p<0.01).

PTZ causes more depleted serotonin stock in the RH, which is a predictor of the convulsive status [9]. Besides, the prolonged administration of PTZ leads to imbalance of amino acids and serotonin in the cerebral hemispheres without preferences toward the LH or the RH.

Compound 78553 significantly enhances and balances the level of GABA, mainly due to increase in the RH. Sodium valproate has a similar localization and direction, but the effect is stronger with preserving the hemispheric asymmetry. As for another inhibitory amino acid – glycine – compound 78553 considerably increases its level compared to sodium valproate. It is important that compound 78553 and the reference drug eliminate the asymmetry appeared as a result of PTZ due to the increased level of glycine preferably in the LH.

Compound 78553 does not restore the balance of glutamate, but eliminates the glutamatergic effect of PTZ in the LH and almost in the RH. The reference drug also restores the balance of amino acid in the cerebral hemispheres, does not compensate the negative effect in the LH, and creates even the negative difference in the RH compared with the intact control.

The aspartate level is fully normalized; almost its normal ratio between the hemispheres is restored when treating with test the compound. Sodium valproate normalizes the aspartate level significantly weaker than compound 78553, but restores its balance in hemispheres.

Compound 78553 normalizes the level of serotonin (more in the RH) significantly exceeding the effect of sodium valproate.

Therefore, the chronic model of epileptogenesis causes a significant imbalance of serotonin and amino acids without a significant preference towards any hemisphere: the levels of GABA, aspartate and serotonin are clearly changed in the RH, and the levels of glycine, glutamate – in the LH. Not statistically significant higher level of glutamate in the LH in norm and its significant increase on the PTZ kindling model may be related to frequent development of epileptic foci in the LH (it is often recorded on the EEG) [4].

Moreover, PTZ kindling depletes the stock of serotonin more than 3 times; its significance in epileptogenesis needs further clarification. There is an evidence of the serotonin effect on epileptohenesis [18]: its reduction leads to the increased convulsive readiness. This may be due to the fact that serotonin interacts with a variety of different receptor subtypes linked to divergent signal transduction cascades, thereby often exerting the opposing control on cell membrane potentials [9]. Thus, the property of AEDs to increase the serotonin level in the brain is one of the ways to achieve better control of convulsive states [18].

There is a consistent pattern when receiving PTZ that compound 78553 is more effective in the hemisphere where deviation of the levels of neurotransmitters from the level of intact control is more expressive. In addition, the test compound better affects the excitatory amino acids, which levels approach to those in the intact control. In chronic epileptogenesis sodium valproate is more effective on inhibitory neurotransmitters and serotonin in those hemispheres, which are strongly affected by PTZ. However, excitatory neurotransmitters do not possess this action. At the same time, in contrast to compound 78553, sodium valproate has the powerful effect on GABA and glutamate, which levels approach the level of intact control. This is important because their balance plays a key role in development of epileptic seizures [19].

Correlation analysis (Tab. 3) has shown that there is no statistically significant relationship between neurotransmitters in the LH of intact mice. There is a significant medium strength positive correlation in the serotonin–GABA relationship and the similar negative relationship between serotonin and glutamate in the RH. In addition, there are a statistically significant strong relationships (r = 0.77-0.97) between the levels of all neurotransmitters in the LH and the RH (except GABA), which indicates conjugation of neurotransmitter processes in different cerebral hemispheres. The balance of transmitters in the hemispheres changes, and a significant correlation between their levels disappears when taking compound 78553. There is a positive correlation ($p < 0.05$) in the glutamate–GABA relationship in the LH and the RH, which reflects stronger conjugation processes in transformation glutamate in GABA [20]. In addition, there is a strong negative relationships of aspartate–GABA (r = -0.85) and aspartate–glutamate (r = -0.77) and a strong positive correlation in the relationships of GABA–glycine (r = 0.88) and glutamate–glycine (r = 0.80) in the RH. A positive correlation between inhibitory amino acids indicates the related increase of their levels in case of administration of compound 78553, and it is important for the anticonvulsant activity. In contrast to compound 78553, sodium valproate destroys relationships of the mediator levels between the hemispheres for GABA and glutamate, keeping a positive correlation only for aspartate and glycine. There is no significant correlation in the LH, but there is the direction change in the serotonin–glutamate relationship (r = 0.85 versus r = -0.64 in intact control) and increase in the relationship strength of serotonin–GABA in the RH. In this hemisphere there is a strong positive correlation (r = 0.87) in relationships of glutamate–GABA and glycine–aspartate. The difference between correlations created by the effect of compound 78553 and sodium valproate indicates the differences in their biochemical mechanisms of the anticonvulsant action.

In case of PTZ kindling only typical positive correlation of the glycine level in the LH and the RH (r = 0.96) retains, other mediators lose their correlations. Chronic epileptogenesis is characterized by a strong negative correlation in the GABA–glutamate relationship. In addition, there is a negative glycine–aspartate relationship in the RH (r = -0.91). Such a negative correlation between inhibitory and excitatory amino acids corresponds to the concept of the E pathogenesis (the levels of excitatory neurotransmitters increase, and the levels of inhibitory ones decrease [1]). The direction in relationships of serotonin–GABA and serotonin–glutamate changes indicating a deep disorder of serotonin–amino acid interactions in the RH.

Compound 78553 with an increase in the level of inhibitory neurotransmitters creates a significant strong correlation between them in both hemispheres. There is a strong positive correlation ($p < 0.05$) between the level of glutamate and inhibitory amino acids (r = 0.94 for GABA, r = 0.91 for glycine) in the RH, which may be important in the biochemical mechanism of the anticonvulsant action of the test compound. Moreover, the relationship between GABA and glutamate disappears in the LH. In case of sodium valproate there is a strong positive relationship (r = 0.99) between the level of GABA in different hemispheres ($p < 0.05$), indicating enhancement of the exchange conjugation of this neurotransmitter. Sodium valproate retains a significant strong negative relationship of GABA–glutamate (r = -0.96) in the LH, and it weakens in the RH (r = -0.66).

Table 3

Statistically significant correlations between the levels of serotonin, inhibitory and excitatory amino acids in separate brain hemispheres on the effect of compound 78553 and sodium valproate in intact mice and the pentylentetrazole kindling model (n = 44)

Group	Intact animals			Pentylentetrazole-induced kindling		
	Intact control	Compound 78553	Sodium valproate	PTZ (control)	PTZ + Compound 78553	PTZ + sodium valproate
LH						
Ser-GABA	–	–	–	–	–	0.93
Ser-Gli	–	–	–	–	–	–
Ser-Glu	–	–	–	–	–	–0.92
Ser-Asp	–	–	–	–	–	–0.90
GABA-Gli	–	–	–	–	0.85	–
GABA- Glu	–	0.78	–	–0.88	–	–0.96
GABA-Асп	–	–	–	–	–	–
Gli-Glu	–	–	–	–	–	–
Gli-Asp	–	–	–	–	–	–
Glu-Asp	–	–	–	–	–	–
RH						
Ser-GABA	0.67	–	0.91	–0.86	–	–
Ser-Gli	–	–	–	–	–	–
Ser-Glu	–0.64	–	0.85	0.71	–	–
Ser-Asp	–	–	–	–	–	–
GABA-Gli	–	0.88	–	–	0.86	–0.84
GABA-Glu	–	0.88	0.87	–0.80	0.94	–
GABA-Asp	–	–0.85	–	–	–	–
Gli-Glu	–	0.80	–	–	0.91	0.85
Gli-Asp	–	–	0.87	–0.91	–	–
Glu-Asp	–	–0.77	–	–	–	–
LH-RH						
Ser	0,86	–	–	–	–	–
GABA	–	–	–	–	–	0.99
Gli	0.85	–	0.93	0.96	–	–
Glu	0.77	–	–	–	–	–
Asp	0.97	–	0.82	–	–0.83	–

Note. Ser – serotonin, GABA – γ-Aminobutyric acid, Gli – glycine, Glu – glutamate, Asp – aspartate, LH – left hemisphere, RH – right hemisphere, PTZ – pentylentetrazole.

The last correlation is not statistically significant. Like compound 78553 sodium valproate forms a significant strong positive correlation (r = 0.85) in glutamate–glycine relationship, and it is negative in the glycine–GABA relationship (r = -0.84) in the RH. In addition, sodium valproate creates a strong positive correlation in the serotonin–GABA relationship (r = 0.96), a negative correlation in the relationships of serotonin–glutamate (r = -0.92) and serotonin-aspartate (-0.90) in the LH (but not in the RH).

Thus, PTZ kindling causes a significant imbalance of neurotransmitters in the brain of mice, increases the levels of excitatory neurotransmitters, at the same time reducing the inhibitory amino acids and depletes the stock of serotonin in both hemispheres. Compound 78553 in case of PTZ kindling clearly increases the serotonin level, moderately increases the levels of GABA and glycine in the brain and reduces the amount of excitatory amino acids almost to the level of intact control. The test compound is more effective in the hemisphere where deviation of inhibitory amino acids is stronger than the level of intact mice under the influence of PTZ. Sodium valproate increases the level of GABA more effectively than compound 78553, however, it has a weaker effect on other inhibitory neurotransmitter – glycine. Regarding excitatory amino acids so-

dium valproate restores the physiological level of glutamate, slightly affecting the level of aspartate. A moderate recovery effect is observed for serotonin in the RH, and it is almost absent in the LH. The glutamate–GABA balance plays an important role in seizures [19]. Sodium valproate has the greatest effect on these mediators, which make it highly effective. The effectiveness of compound 78553 is equal to sodium valproate in the PTZ kindling model [12], and it may be related to a stronger effect on the metabolism of serotonin and glycine. In intact mice neurotransmitters are in balance between the hemispheres; in addition, serotonin is characterized by the hemispheric asymmetry in norm and on the epileptogenesis model. Compound 78553 and sodium valproate causes the hemispheric asymmetry for different amino acids in intact animals. PTZ kindling causes imbalance of all amino acids, compound 78553 restores a balance of inhibitory neurotransmitters and sodium valproate – of glycine and aspartate.

CONCLUSIONS

1. The hemispheric asymmetry of the level of excitatory and inhibitory amino acids is absent in intact mice. 1-(4-Metoxyphenyl)-5-{2-[4-(4-metoxyphenyl) piperazine-1-yl]-2-oxoethyl}-1,5-dihydro-4H-pyrazole[3,4-d] pyrydine-4-one does not affect the levels of GABA and glutamate, but strongly increases the amount of glycine in both hemispheres (more in the left one) and moderately – of aspartate (equally), retains the hemispheric symmetry of amino acids, except glycine. Sodium valproate strongly increases the level of GABA and moderately reduces the level of glutamate mainly in the right hemisphere.

2. The level of serotonin in the left hemisphere of intact mice is higher than in the right one. 1-(4-Metoxyphenyl)-5-{2-[4-(4-metoxyphenyl)piperazine-1-yl]-2-oxoethyl}-1,5-dihydro-4H-pyrazole[3,4-d] pyrydine-4-one moderately increases the levels of serotonin mainly in the right hemisphere, and sodium valproate, conversely, decreases in the left hemisphere.

3. Pentylenetetrazole-induced kindling causes a reduction of inhibitory neurotransmitters in the brain and an increase of excitatory ones. In addition, the hemispheric asymmetry of the levels of all amino acids appears. In this case, 1-(4-metoxyphenyl)-5-{2-[4-(4-metoxyphenyl) piperazine-1-yl]-2-oxoethyl}-1,5-dihydro-4H-pyrazole[3,4-d] pyrydine-4-one effectively reduces the level of excitatory neurotransmitters (mainly in the left hemisphere for aspartate and in the right hemisphere for glutamate), their amount approach to the normal level, restores the symmetry of inhibitory amino acids between the hemispheres. Sodium valproate reduces the level of glutamate and increases the level of GABA mainly in the right hemisphere, restores the hemispheric balance for glycine and aspartate.

4. Pentylentetrazole asymmetrically depletes the serotonin pool mainly in the right hemisphere. 1-(4-Metoxyphenyl)-5-{2-[4-(4-metoxyphenyl)piperazine-1-yl]-2-oxoethyl} -1,5-dihydro-4H-pyrazole [3,4-d]pyrydine-4-one more effectively restores the level of serotonin than sodium valproate. Serotonergic effects of drugs are mainly localized in the right hemisphere.

5. Correlation analysis indicates that there is the conjugation deviation of the neurotransmitter exchange for acids and serotonin after long-term PTZ administration. The changes in direction and the strength of relationships between the neurotransmitter levels indicate the differences between biochemical mechanisms of the anticonvulsant action of compound 78553 and sodium valproate.

Conflicts of Interest: authors have no conflict of interest to declare.

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Надійшла до редакції 20.01.2017 р.