I. N. Tyurenkov, V. N. Perfilova, L. I. Mikhailova, G. A. Zhakupova, S. A. Lebedeva

Volgograd State Medical University, Russian Federation

Comparative Study of the Effects of New Neuroactive Amino Acid Derivatives on the Postnatal Development of the Rat's Offspring with Experimental Preeclampsia

Background*: Our aim was to investigate the effect of derivatives of GABA and glutamate on the postnatal development of the offspring of rats with experimental preeclampsia.* ***Methods****: The experiments were performed on 35 albino female rats aged 5–7 months, weighing 220–240 g, and their offspring in the amount of 284 individuals. Experimental preeclampsia was modeled by replacing the drinking water by 1.8% NaCl solution to pregnant females from 7 to 21 days of gestation. Glutamic acid — compound RSPU-135 at a dose of 26 mg/kg, GABA derivative — compound RSPU-242 at a dose of 23 mg/kg and the reference drug sulodexide in a dose of 30 mg/kg administered to female orally daily, since the 7th day of gestation prior to delivery. Evaluated the physical development of offspring, sensory-motor reflexes, mental functions.* ***Results****: It was found that the experimental preeclampsia causes a delay in physical development and maturation of sensory-motor reflexes in the offspring, as indicated by the later periods of eruption of the incisors and eye opening, response to the emergence of audio and olfactory stimuli, forming vestibular stability and coordination of movements compared to pups from females with physiological pregnancy. Offspring from females with experimental preeclampsia were noted for lagging behind in mental development, as evidenced by the decline of the orienting-exploratory activity, learning and memory, increase of anxiety level. Compound RSPU-135, to a greater extent, improves physical development, increases the rate of maturation of sensory-motor reflexes, RSPU-242 — stimulation of cognitive functions, keeping the memory trace, orienting-exploratory, spontaneous locomotor activity, and reduce of anxiety level.* ***Conclusion:*** *The neuroactive amino acid derivatives limit the negative effects of experimental preeclampsia on the offspring.*

Key words: *experimental preeclampsia, postnatal development, derivatives of neuroactive amino acid.*
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Background

Preeclampsia is one of the most prevalent pregnancy complications. It prevalence is 7-22% and has been increasing in the past years. This symptom cluster is

considered to be the major cause of perinatal mortality (2-3%) and neonatal morbidity (about 70%) [1, 2]. The main factors underlying preeclampsia pathogenesis include vascular endothelium dysfunction resulting in decreased vasodilating, antithrombotic, anti-inflammatory and antiproliferative functions. All these lead to impaired fetal placental blood circulation, hypoxia, fetal developmental delay and sometimes to embryonic death [3, 4]. Hypoxic damage to the central nervous system results in a wide range of long-term mental health disorders in children: attention deficit syndrome, cerebral palsy with motor, cognitive, communication and intellectual impairments, vegetative and hemoliquorodynamic disorders in adolescence [5-7]. These facts highlight the significance of a search for agents which will prove effective in preventing preeclampsia and reduce its negative outcomes for the fetus.

A number of studies demonstrated that neuroactive amino acid derivatives (NAAD), namely, glutamate and GABA derivatives, have vasodilating, anticoagulant and antihypoxic effects as well as normalize microcirculation [8]. Their findings make it possible to hypothesize that NAAD have gravidoprotective properties.

The purpose of this study was to comparatively explore the effects of hydrochloride-3-phenyl-glutamic acid (a compound whose laboratory code is RSPU-135) and hydrochloride of methyl ester 3 (pyridil-3)-GABA (a compound whose laboratory code is RSPU-242) on postnatal development of the offspring of rats with experimental preeclampsia (EP).

Methods

Study Design

We modeled experimental preeclampsia by replacing drinking water with 1.8% NaCL solution from the 7^{th} to 21^{st} days of gestation [9].

Intervention Description

The indicators of physical development of the offspring (eruption of incisors, the moment the pups first opened their eyes) were registered visually.

The development of vestibular reactions and coordination of movements was observed using the cliff avoidance and aerial righting reflex tests; the development of muscular power and tone was elicited by means of a horizontal wire test; we also registered their first reaction to an olfactory stimulus as well as to an auditory stimulus.

Cliff avoidance test: the pups were placed on the table so that their forelegs could touch the edge of the table. They were considered to have developed the reflex if they could crawl away from the edge in 10s [10].

Aerial righting reflex test: the pups were held upside down at a height of 60 cm over a soft surface and then were quickly dropped. Their body torsion and falling on four legs were registered [10].

Muscular power was assessed using a horizontal wire test. The animal was placed on a dense horizontal wire which was immediately rotated by 180 degrees. The time which passed from the moment the animal was placed on the wire till the moment it fell from it was registered [10].

Reaction to an auditory stimulus: the pups were placed in a soundproof chamber in a cage. They were considered to have developed the reflex if the animal could react to an auditory stimulus lasting 0.3s [10].

The mental functions of the offspring of female rats with preeclampsia were evaluated in the juvenile period of postnatal ontogenesis using generally accepted tests: open field test (on the 40^{th} day), raised cross-shaped labyrinth test (RCSL) (on the 70^{th} day), conditioned passive avoidance test (CPA; on the 60^{th} day), extrapolation escape test (EET; on the 70^{th} day) [10].

Groups under Study

The animals were grouped in the following way: positive control group (pregnant female rats without EP and their offspring); negative control group (pregnant female rats with EP receiving a saline solution and their offspring); experimental group 1 (pregnant female rats with EP receiving RSPU-135 at a dose of 26 mg/kg and their offspring); experimental group 2 (pregnant female rats with EP receiving RSPU-242 at a dose of 23 mg/kg and their offspring); experimental group 3

pregnant female rats with EP receiving sulodexide, a comparator drug, at a dose of 30 mg/kg and their offspring). The agents were administered orally daily from the 7th day of gestation up to delivery

Ethics Review

The experiments were conducted in accordance with the ethics review (record 176-2013 of May 8, 2013).

Statistical Analysis

Study participants

The experiments were performed on 35 albino outbred female rats aged 4-5 months weighing 220-240g and their offspring (284 individuals) who were kept in the vivarium in the natural daylight and had free access to water and food in the autumn-winter period.

Main Outcomes of the Study

The analysis of the physical development and that of sensory motor reactions has revealed a considerable delay in the offspring of female rats with EP as compared to the group of pups delivered by animals with an uncomplicated pregnancy (charts 1-4, fig. 1). The offspring of females with EP who had received RSPU-135 and RSPU-242 during pregnancy had earlier incisor eruption (see chart 1), they also began to open their eyes (see chart 2), developed vestibular reactions – cliff avoidance test (see chart 3), aerial righting reflex test (see chart 4) and reactions to an auditory stimulus (chart 5) and an olfactory stimulus (chart 6) as well as muscular power and tone – horizontal wire test (see fig.1) earlier. Moreover, a number of indicators in the pups delivered by female rats with EP who had received RSPU-135 exceeded those of the pups of females with EP who had received RSPU-242 during pregnancy.

Chart 1. Terms of incisor eruption in the offspring of female rats with experimental preeclampsia (EP) who received RSPU-135, RSPU-242 and sulodexide from the 7th up to the 21st day of gestation

Animal group	Number of pups with erupted incisors, %

	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day
Offspring of females without EP (n=49)	4,1	12,4	53,1	87,8	100	---	---	---
Offspring of females with EP + saline solution (n=62)	11,3	33,8	67,7	83,9	87,1# (p=0, 020)	90,3	95,1	100
Offspring of females with EP + RSPU-135 (n=49)	8,2	26,5	61,2	91,8	100* (p=0, 020)	---	---	---
Offspring of females with EP + RSPU-242 (n=60)	21,7	40,7	76,3	96,6* (p=0, 045)	100* (p=0,020)	---	---	---
Offspring of females with EP + sulodexide (n=64)	18,8	54,7#	78,1	87,5	100* (p=0, 020)	---	---	---

Note. # - changes are statistically significant compared to the indicator of the offspring of females without EP based on the Fisher criterion

- changes are statistically significant compared to the indicator of the offspring of females with EP based on the Fisher criterion

Chart 2. Terms when the offspring of females with experimental preeclampsia (EP) who had received RSPU-135, RSPU-242 and sulodexide from the 7th up to the 21st day of gestation began to open their eyes

Animal group	**Number of pups who began to open their eyes, %**						
	13th day	**14th day**	**15th day**	**16th day**	**17th day**	**18th day**	**19th day**
Offspring of females without EP (n=49)	0	8,2	40,8	85,7	95,9	100	---
Offspring of females with EP + saline solution (n=61)	0	3,3	19,7# (p=0,020)	36,7# (p=0,000)	53,3# (p=0,000)	80# (p=0,001)	100

Offspring of females with EP + RSPU-135 (n=49)	4,0	18,4*	44,9* (p=0,004)	63,2* (p=0,007)	100* (p=0,015)	---	---
Offspring of females with EP + RSPU-242 (n=59)	0	1,7	23,7	69,5* (p=0,005)	98,3* (p=0,001)	100# (p=0,001)	---
Offspring of females with EP + sulodexide (n=52)	0	1,6	25,4	60,3* (p=0,006)	80,6* (p=0,020)	98,4* (p=0,002)	100

Note. # - changes are statistically significant compared to the indicator of the offspring of females without EP based on the Fisher criterion

- changes are statistically significant compared to the indicator of the offspring of females with EP based on the Fisher criterion

Chart 3. Terms of vestibular reaction development (cliff avoidance test) in the offspring of females with experimental preeclampsia (EP) who received RSPU-135, RSPU-242 and sulodexide from the 7th up to the 21st day of gestation

Animal group	**Number of pups who coped with the test, %**				
	6th day	**7th day**	**8th day**	**9th day**	**10th day**
Offspring of females without EP (n=49)	89,8	100	---	---	---
Offspring of females with EP + saline solution (n=61)	55,7# (p=0,001)	81,9# (p=0,001)	86,8	93,5	100
Offspring of females with EP + RSPU-135 (n=49)	75,5* (p=0,009)	89,8	100* (p=0,083)	---	---
Offspring of females with EP + RSPU-242 (n=60)	80* (p=0,062)	88,1	98,3* (p=0,034)	100	---
Offspring of females with EP + sulodexide (n=64)	85,5* (p=0,001)	100* (p=0,001)	---	---	---

Note. # - changes are statistically significant compared to the indicator of the offspring of females without EP based on the Fisher criterion

- changes are statistically significant compared to the indicator of the offspring of females with EP based on the Fisher criterion

Chart 4. Development of a vestibular function in the offspring of females with experimental preeclampsia (EP) who received RSPU-135, RSPU-242 and sulodexide from the 7th up to the 21st day of gestation during aerial righting reflex test

Animal group	**Number of pups who coped with the test, %**			
	17th day	**18th day**	**19th day**	**20th day**
Offspring of females without EP (n=49)	97,9	100	---	---
Offspring of females with EP + saline solution (n=60)	67,7# (p=0,001)	78,3# (p=0,000)	86,7	100
Offspring of females with EP + RSPU-135 (n=49)	97,9*	100* (p=0,000)	---	---
Offspring of females with EP + RSPU-242 (n=59)	86,4* (p=0,001)	100* (p=0,000)	---	---
Offspring of females with EP + sulodexide (n=62)	96,8* (p=0,004)	100* (p=0,000)	---	---

Note. # - changes are statistically significant compared to the indicator of the offspring of females without EP based on the Fisher criterion

- changes are statistically significant compared to the indicator of the offspring of females with EP based on the Fisher criterion

Chart 5. Terms when the offspring of females with experimental preeclampsia (EP) who had received RSPU-135, RSPU-242 and sulodexide from the 7th up to the 21st day of gestation developed a reaction to an auditory stimulus

Animal group	**Number of pups who coped with the test, %**					
	8th day	**9th day**	**10th day**	**11th day**	**12th day**	**13th day**
Offspring of females without EP (n=49)	77,5	93,8	100	---	---	---
Offspring of females with EP + saline solution (n=61)	31,1# (p=0,000)	37,7# (p=0,000)	44,3# (p=0,000)	80,2	83,6	100
Offspring of females with EP + RSPU-135 (n=49)	93,8* (p=0,000)	95,9* (p=0,000)	100* (p=0,000)	---	---	---

Offspring of females with EP + RSPU-242 (n=59)	67,8* (p=0,001)	91,5* (p=0,000)	98,3* (p=0,000)	100* (p=0,001)	---	---
Offspring of females with EP + sulodexide (n=64)	56,2	81,5* (p=0,000)	98,4* (p=0,000)	100* (p=0,001)	---	---

Note. # - changes are statistically significant compared to the indicator of the offspring of females without EP based on the Fisher criterion

- changes are statistically significant compared to the indicator of the offspring of females with EP based on the Fisher criterion

Chart 6. Terms when the offspring of females with experimental preeclampsia (EP) who had received RSPU-135, RSPU-242 and sulodexide from the 7th up to the 21st day of gestation developed a reaction to an olfactory stimulus

Animal group	**Number of pups who coped with the test, %**							
	10th day	**11th day**	**12th day**	**13th day**	**14th day**	**15th day**	**16th day**	**17th day**
Offspring of females without EP (n=49)	18,3	51,0	87,7	95,9	100	---	---	---
Offspring of females with EP + saline solution (n=61)	26,2	34,4	52,4# (p=0,001)	53,7# (p=0,000)	65* (p=0,000)	83,3	98,3	100
Offspring of females with EP + RSPU-135 (n=49)	22,4	40,8	60,4	77,1* (p=0,041)	83,3* (p=0,048)	100* (p=0,002)	---	---
Offspring of females with EP + RSPU-242 (n=59)	20,3	38,9	66,1	74,5	86,4* (p=0,009)	93,2	100	---
Offspring of females with EP + sulodexide (n=63)	33,4	44,4	73,0* (p=0,025)	88,8* (p=0,001)	96,8* (p=0,000)	100* (p=0,001)	---	---

Note. # - changes are statistically significant compared to the indicator of the offspring of females without EP based on the Fisher criterion

- changes are statistically significant compared to the indicator of the offspring of females with EP based on the Fisher criterion

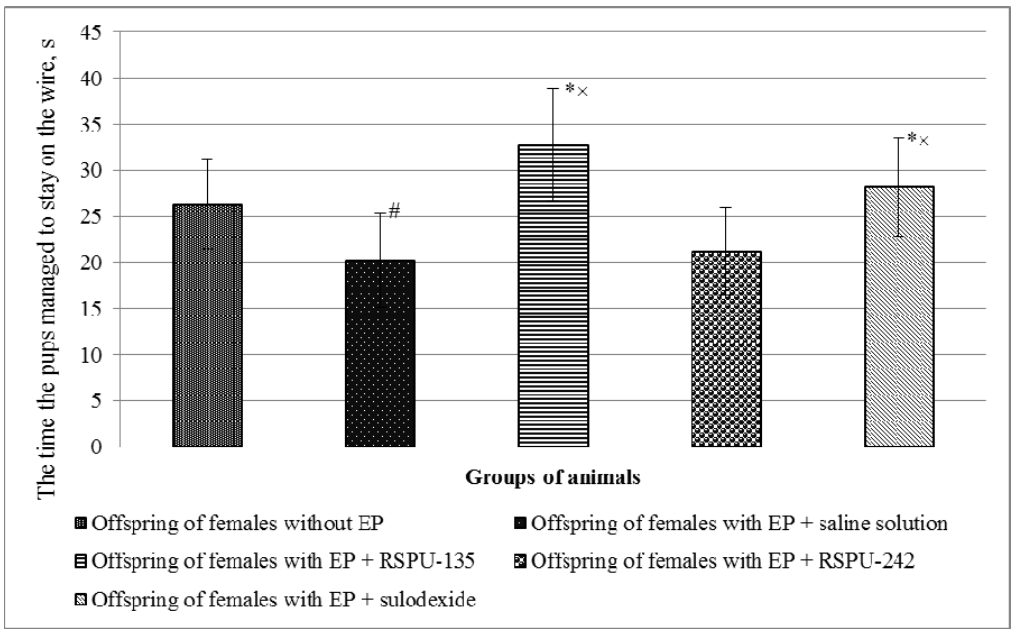

Fig 1. The time the offspring of females with EP who had received RSPU-135, RSPU-242 and sulodexide from the 7th up to the 21st day of gestation managed to stay on the horizontal wire.

Note:# p= 0.006 – changes are statistically significant compared to the indices of the offspring of females without EP (Kruskal-Wallis, Siegel-Castellan criterion), * p=0.003 – changes are statistically significant compared to the indices of the offspring of females with EP (Kruskal-Wallis, Siegel-Castellan criterion), × p=0.04 – changes are statistically significant compared to the indices of the offspring delivered by females with EP + RSPU-242 (Kruskal-Wallis, Siegel-Castellan criterion).

The open field test revealed that locomotor, orientation and exploratory activity of the pups of the female rats with EP was lower than that of the offspring of the females with an uncomplicated pregnancy which was proved by a smaller number of squares crossed by them (p=0.000), the amount of cases of rearing (standing on their hind legs) and peeping into a hole (p=0.009). The number of boluses in the pups from the negative control group was higher (p=0.011), however, the number of short time grooming acts was statistically insignificantly lower than that of the offspring of the positive control group which indicates a dissociation between their emotional state and vegetative manifestations. The pups of the females with

EP who had received RSPU-242 and sulodexide exhibited higher motor and exploratory activity as well as fewer fecal boluses compared to the negative control group. Horizontal motor activity alongside with orientation and exploratory activity was slightly lower in the animals of the 1st experimental group (p=0.002) than those in the 2nd experimental group (chart 7).

The RCSL test demonstrated that on the 70th day of the postnatal period the pups delivered by females with EP spent less time in the open arms compared to the positive control group (0.000). Fewer cases of hanging over the edge of the open arms (p=0.000) were also observed. The pups of females with EP who had received RSPU-242 and sulodexide during gestation spent more time in the open arms and less time in the closed arms of the labyrinth which indicates a lower anxiety level in the pups of this group compared to the negative control group.

Chart 7. Behavioural parameters of the offspring of rats with experimental preeclampsia (EP) who received RSPU-135, RSPU-242 and sulodexide in the open field test on the 40th day of their postnatal period

Animal group	**HMA**	**OEA**	**Time spent in the CZ, s**	**Number of entries into the CZ**	**Short term grooming**	**Boluses**
Offspring of females without EP (n=48)	47,6±9,5	19,5±4,8	5,3 ±1,6	1,5 ±0,8	2,5±1,2	2,0±1,0
Offspring of females with EP + saline solution (n=58)	35,5±8,7* (p=0,000)	16,7±2,6* (p=0,009)	3,1 ±0,8* (p=0,020)	0,6 ±0,5* (p=0,000)	2,1 ±0,9	2,6±0,7* (p=0,011)
Offspring of females with EP + RSPU-135 (n=46)	38,5 ±7,4	16,3 ±2,6	2,2 ±0,9 $^{\#}$ (p=0,035)	0,5 ±0,5	2,3 ±0,9	1,9±0,6 $^{\#}$ (p=0,000)
Offspring of females with EP + RSPU-242 (n=47)	46,5±10,9 $^{\#\wedge}$ (p=0,000)	19,3±2,8 $^{\#\wedge}$ (p=*0,012*)	3,5±1,3$^{\wedge}$ (p=0,015)	0,8 ±0,4	1,5±0,6$^{\#\wedge}$ (p=0,046)	2,1±0,3$^{\#}$ (p=0,046)
Offspring of females with EP + sulodexide (n=52)	42,3 ±9,3$^{\#}$ (p=0,001)	18,7±4,4	2,4±0,4$^{\#\times}$ (p=0,030)	0,7 ±0,5	3,1±0,9$^{\#\wedge\times}$ (p=0,000)	1,9±0,3$^{\#}$ (p=0,000)

Note. HMA - horizontal motor activity, OEA - orientation and exploratory activity, CZ- central zone. * - changes are statistically significant according to the Kruskal-Wallis criterion compared to the offspring of females without EP, # - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP, ˆ - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP +RSPU-135, × - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP + RSPU-242.

Chart 8. Behavioural parameters of the offspring of rats with experimental preeclampsia (EP) who received RSPU-135, RSPU-242 and sulodexide in the raised cross-shaped labyrinth test on the 70th day of their postnatal period

Animal group	**Number of open arm entries**	**Time spent in OA, s**	**Cases of hanging over the edge in OA**	**Closed arm entries**	**Time spent in CA, s**
Offspring of females without EP (n=48)	2,6±0,7	50,4±11,6	5,5 ±1,5	3,4 ±1,0	105,5 ±9,9
Offspring of females with EP + saline solution (n=49)	3,1±0,9 $^{*\wedge}$ (p=0,014)	38,5±8,4** (p=0,000)	3,9 ±1,5* (p=0,000)	3,9±0,9* (p=0,004)	116,5±13,9** (p=0,000)
Offspring of females with EP + RSPU-135 (n=39)	2,3 ±0,6	37,5 ±5,2	3,9 ±1,2	3,7 ±0,5	118,6 ±7,2
Offspring of females with EP + RSPU-242 (n=42)	3,3±1,1$^{\wedge}$ (p=0,000)	52,6±11,7$^{\#\#\wedge\wedge}$ (p=0,000)	5,0±1,8 $^{\#\wedge}$ (p=0,000)	3,7 ±0,9	99,4±12,7$^{\#\#\wedge\wedge}$ (p=0,000)
Offspring of females with EP + sulodexide (n=51)	2,9 ±0,9$^{\wedge}$ (p=0,004)	45,6±13,9$^{\#\#\wedge\wedge\times\times}$ (p=0,000)	4,5 ±1,7	3,7 ±1,3	108,7±22,8$^{\#\#\wedge\wedge\times\times}$ (p=0,000)

Note. CA – closed arms, OA – open arms, * - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females without EP, ** - changes are statistically significant according to the Newman-Keuls criterion compared to the offspring of females without EP, # - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP, ## - changes are

statistically significant according to the Newman-Keuls criterion compared to the offspring of females with EP , ˆ - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP + RSPU-135, ˆˆ - changes are statistically significant according to the Newman-Keuls criterion compared to the offspring of females with EP + RSPU-135, × - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP + RSPU-242, ×× - changes are statistically significant according to the Newman-Keuls criterion compared to the offspring of females with EP + RSPU-242.

No differences between the behaviour of the pups of females who had received RSPU-135 and that of the negative control group were registered (chart 8).

During the CPA test on the 60^{th} day of the postnatal period after a memory trace was created and checked on the 1^{st}, 3^{rd}, 7^{th} and 14^{th} day of training the percentage of the pups of females with EP entering a dark chamber was insignificantly higher than that in the positive control group. RSPU-242 yielded improvement in long-term memory of the offspring of females with EP which manifested itself as a longer latency period before entering a dark area ($p=0.004$) as well as decreased number of pups who entered a dark chamber when the skill was activated and less time spent in it ($p=0.000$)

The strength of the memory trace in the offspring of females with EP who had received RSPU-135 was similar to that in the negative control group (chart 9).

In the extrapolation escape test on the 70^{th} day of the postnatal period the percentage of the pups who coped with the task of escaping an aversive environment was lower in the group with EP compared to the positive control group both during training and activation of the skill. In all the experimental groups the number of individuals who managed the task was higher compared to the group of the offspring of females with EP who had received a saline solution. At the training stage a latency period of diving underwater was shorter in the offspring of rats who had received RSPU-242 during pregnancy than in the pups

delivered by females who had received RSPU-125 and sulodexide. When the skill was activated the latency period before diving was practically the same in all the experimental groups but shorter than that in the negative control group which may indicate a higher rate of orientation reactions in them (chart 10).

Discussion

The problem of preeclampsia which is one of the causes of perinatal disorders in mothers, embryos and neonates yielding unfavourable outcomes for their health in the postnatal period and afterlife remains extremely important which is proved by its high prevalence (16-21%) [11-13].

At present endothelial dysfunction is considered to play a key role in the pathogenesis of preeclampsia [11, 14, 15]. It can be triggered by oxidative stress, hypoxia, hyperhomocysteinemia and other factors [14,16]. Endothelial dysfunction is characterized by generalized vasospasm, hypercoagulation, worsened oxidative stress, placental hypoxia and/or ischemia resulting in various disorders both in mothers and developing embryos [17].

Chart 9. Behavioural parameters of the offspring of rats with experimental preeclampsia (EP) who received RSPU-135, RSPU-242 and sulodexide in the conditioned passive avoidance test on the 60th day of their postnatal period

Time	**Indicators**	**Group (M ± σ)**				
		Offspring of females without EP (n=37)	**Offspring of females with EP + saline solution (n=45)**	**Offspring of females with EP + RSPU-135 (n=34)**	**Offspring of females with EP + RSPU-242 (n=38)**	**Offspring of females with EP + sulodexide (n=55)**
Training	LP before entering DC, s	38,6 ±10,1	39,8±9,6	50,5 ±13,4	44,2 ±9,7	34,7 ±7,4
Activation 1st day	LP before entering DC, s	180,0	180,0	175, 1±6,1* (p=0,002)	180,0$^{\wedge}$ (p=0,004)	177,3 ±7,1$^{\#\times}$ (p=0,002)

	Time spent in DC, s	0	0	3, 1±1,2* (p=0,000)	0^ (p=0,000)	2,0 ±0,9 #× (p=0,000)
	Number of entries	0	0	0,09 ±0,3	0	0,03 ±0,2
	Number of pups entering DC, %	0	0	6	0	2
Activation 3rd day	LP before entering DC, s	178,4±7,3	170, 0±25,3* (p=0,003)	166,5 ±19,5	180,0#^ (p=0,000)	169,1±19,3#× (p=0,046)
	Time spent in DC, s	0,4±0,4	6,2±2,2* (p=0,000)	6,6±1,5	0#^ (p=0,000)	10,2±4,0#^× (p=0,007)
	Number of entries	0,1±0,3	0,1±0,3	0,09 ±0,3	0	0,1 ±0,5
	Number of pups entering DC, %	5	7	9	0	7
Activation 7th day	LP before entering DC, s	176,2±16,5	166,6±22,8	167,7 ±21,4	174,6 ±6,6	159,2±30,6^× (p=0,001)
	Time spent in DC, s	1,8±0,4	6, 8±1,9* (p=0,001)	8,1±1,9	0,2 ±0,4#^ (p=0,000)	17,1±4,0#^× (p=0,000)
	Number of entries	0,1±0,3	0,2±0,4	0,2±0,4	0,1 ±0,2	0,2±0,4
	Number of pups entering DC, %	5	9	9	5	18
Activation 14th day	LP before entering DC, s	169,8±21,0	162,9±30,7	156,1±24,5	169,3 ±15,1	141,6±35,9# (p=0,014)
	Time spent in DC, s	3,9±1,6	12, 9±4,2* (p=0,000)	14, 9±2,5	6,9 ±1,9#^ (p=0,007)	30,2±9,8#^× (p=0,000)
	Number of entries	0,1±0,3	0,2±0,4	0,3 ±0,5	0,2 ±0,4	0,5 ±0,6
	Number of pups entering DC, %	8	11	18	7	25

Note. LP- latency period; DC – dark chamber. - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females without EP, # - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP, ˆ - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP + RSPU-135, × - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP + RSPU-242.

The earlier studies demonstrated that modeling experimental preeclampsia by replacing drinking water consumed by female rats with 1.8% sodium chloride solution during gestation results in elevated concentration of homocysteine in blood plasma [18], increased content of lipid peroxidation (LP) products in the uterus, placenta, brain and liver [19, 20], development of endothelial dysfunction, decreased uterine placental blood flow and intrauterine hypoxia [21]. Impaired blood circulation in the mother-placenta-embryo system as well as hypoxia caused by it are likely to account for a delay in postnatal development which has been proved by the facts that the incisors of the offspring of female rats with EP erupt later, they also begin to open their eyes, develop vestibular reactions, coordination of movements, muscular power and tone as well as cognitive functions at a later time as compared to the pups of females with an uncomplicated pregnancy.

RSPU-135 and RSPU-242 have proved to restrict harmful effects of EP on the offspring. Earlier physical development as well as development of sensory motor systems and vestibular function was observed in the pups of females who had received RSPU-135. RSPU-242 mostly has a positive effect on the psycho-emotional state of the offspring, their cognitive functions and decreases their anxiety level. It is probable that RSPU-135 and RSPU-242 improve endothelial function in female rats with EP. When continuously exposed to damaging factors

endothelium has been reported to struggle to maintain intravascular homeostasis stability. Therefore, under such unfavourable conditions there is a constant need for agents promoting vasodilation, preventing hypercoagulation and hyperaggregation, having anti-inflammatory and antioxidant activity. Eventually, these factors may bring about an imbalance when vasoconstrictive, prothrombogenic and proinflammatory endothelial factors start to prevail which has been proved by the findings on the functional state of endothelium in different diseases obtained by a number of researchers [22-24]. GABA derivatives have been reported to have vasodilating and antiaggregation effects as well as to inhibit LP which may together account for their endothelium protective activity [25, 26]. This may possibly result in enhanced uterine placental blood flow, better oxygen supply to the fetus which promotes trophism and development of various organs and tissues including brain. As we have earlier demonstrated GABA derivatives similar to the agents under study prevent an elevation in the homocysteine content which is known as a neurotoxin capable of having a negative effect on the intrauterine development of brain in female rats with EP [18].

Chart 10. Behavioural parameters of the offspring of rats with experimental preeclampsia (EP) who received RSPU-135, RSPU-242 and sulodexide in the extrapolation escape test on the 70th day of their postnatal period

Time	**Indicators**	**Group (M ± σ)**				
		Offspring of females without EP (n=48)	**Offspring of females with EP + saline solution (n=49)**	**Offspring of females with EP + RSPU-135 (n=42)**	**Offspring of females with EP + RSPU-242 (n=48)**	**Offspring of females with EP + sulodexide (n=60)**
Training	LP before motor activity, s	7,5±1,1	7,7±1,5	4,8±1,1$^{\#}$ (p=0,000)	7,1±1,5$^{\wedge}$ (p=0,000)	6,6±1,3$^{\#\wedge}$ (p=0,001)
	LP of diving	65,6±4,3	100,9±22,4* (p=0,000)	95,6±15,8	72,4±14,3$^{\#\wedge}$	94,6±9,0$^{\#\times}$ (p=0,000)

	underwater, s					
	% of pups who coped with ET	79	49	64	73	55
Activation 1st day	LP before motor activity, s	6,7±1,9	6,9±1,8	5,0±1,4# (p=0,000)	7,8±1,9^ (p=0,000)	6,1±1,6× (p=0,000)
	LP of diving underwater, s	55,2±8,2	88,5±11,3* (p=0,000)	65,9±7,4# (p=0,000)	71,6±12,1#^ (p=0,000)	83,1±10,1#^× (p=0,000)
	% of pups who coped with ET	79	59	71	71	65
Activation 3rd day	LP before motor activity, s	6,1±1,5	11,7±2,4* (p=0,000)	10,8±2,4	7,8±1,9#^ (p=0,000)	9,3±1,9#^× (p=0,000)
	LP of diving underwater, s	36,9±9,4	82,1±11,1*	56,9±6,5# (p=0,001)	61,7±9,9# (p=0,000)	59,3±11,6# (p=0,001)
	% of pups who coped with ET	85	57	74	71	73
Activation 7th day	LP before motor activity, s	12,1±2,5	16,1±4,8* (p=0,008)	10,1±1,6# (p=0,00)	8,5±1,9#	11,1±2,8#× (p=0,000)
	LP of diving underwater, s	33,2±6,3	62,0±12,4* (p=0,000)	42,9±5,8# (p=0,000)	44,1±7,2# (p=0,000)	54,0±8,6^× (p=0,000)
	% of pups who coped with ET	88	71	81	79	73
Activation 14th day	LP before motor activity, s	12,7±3,1	11,5±2,7	14,8±1,5# (p=0,000)	12,4±2,8^ (p=0,001)	14,9±2,6#× (p=0,001)
	LP of diving underwater, s	23,4±5,8	55,2±11,2* (p=0,00)	42,3±7,8# (p=0,000)	35,4±5,4# (p=0,000)	48,3±10,3^× (p=0,022)
	% of pups	94	78	83	88	78

	who coped with ET					

Note. LP- latency period, ET – extrapolation task. - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females without EP, # - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP, ˆ - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP + RSPU-135, × - changes are statistically significant according to the Kruskal-Wallis, Siegel-Castellan criterion compared to the offspring of females with EP + RSPU-242.

As is known, GABA and its derivatives have a nootropic effect, increase glucose utilization and tissue respiratory activity, activate life energy processes, higher integrative functions of the brain. They also have a favourable effect on learning, moderate psychostimulating and antihypoxic effects and enhance memory [27]. Polivalent action of GABA and glutamic acid derivatives can possibly account for reduced damaging effects of preeclampsia on the development of the offspring.

The obtained findings enabled us to make a conclusion that a further search for new highly effective and safe agents among neuroactive amino acid derivatives to prevent and treat preeclampsia as well as to correct postnatal development disorders is expedient.

Conclusion

EP induced by replacement of drinking water with 1.8 sodium chloride solution results in inhibited development of sensory motor reflexes as well as delayed physical development, increases anxiety level and has a negative effect on the cognitive functions of the offspring. Higher indicators of physical development, vestibular and sensory functions were observed in the pups of female rats with EP who had received RSPU-135, a glutamic acid derivative, during pregnancy

compared to the pups of females with EP of the control group and those who had received RSPU-242, a GABA derivative. Moreover, the indices of cognitive functions, orientation and exploratory behaviour in the offspring of females with EP who had been given RSPU-242 during pregnancy exceeded those in the control group as well as in the group which had received RSPU-135.

The findings of the study have proved that an in-depth study of pharmacological activity of RSPU-135 and RSPU-242 and subsequent development of new drugs with a polytropic pathogenetic mechanism of action reducing damaging effects of preeclampsia on the mother and fetus on their basis is very promising.

Conflict of Interest

The authors of this article confirm that no financial support has been provided to them (no conflict of interest) which needs to be reported.

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CONTACT INFORMATION

Tyurenkov Ivan Nikolaevich, PhD, professor, associate fellow of the RAS, head of the department of pharmacology and biopharmacy of the faculty for continuing medical education of VolgSMU.

Address: 3a, Pugatcheyovskaya Street, Volgograd, 400001, **tel.:** + 7 (8442) 97-81-80, **e-mail:** fibfuv@mail.ru

Perfilova Valentina Nikolaevna, PhD senior research associate of the laboratory of pharmacology of cardiovascular agents of the research institute of pharmacology of VolgSMU.

Address: 3a, Pugatcheyovskaya Street, Volgograd, 400001, **tel.:** + 7 (8442) 97-81-80, **e-mail:** vnperfilova@mail.ru

Mikhailova Lyudmila Ivanovna, postgraduate student of the department of pharmacology and biopharmacy of the faculty for continuing medical education of VolgSMU.

Address: 3a, Pugatcheyovskaya Street, Volgograd, 400001, **tel.:** + 7 (8442) 97-81-80, **e-mail:** mih-li@mail.ru

Zhakupova Gulnara Aleksandrovna, MD, doctoral researcher of the department of pharmacology and biopharmacy of the faculty for continuing medical education of VolgSMU.

Address: 3a, Pugatcheyovskaya Street, Volgograd, 400001, **tel.:** + 7 (8442) 97-81-80, **e-mail:** g.a.zhakupova@yandex.ru

Lebedeva Svetlana Aleksandrovna, MD, senior research associate of the laboratory of synthesis of antiviral agents of the research institute of pharmacology of VolgSMU.

Address: 3a, Pugatcheyovskaya Street, Volgograd, 400001, **tel.:** + 7 (8442) 97-81-80, **e-mail:** lebedeva.farm@mail.ru