QUANTITATIVE CHANGES OF LIPOFUSCIN UNDER FOCAL PERMANENT ISCHEMIA CONDITIONS OF BRAIN AFTER ADMINISTRATION OF 5-HYDROXYADAMANTANE-2-ON, GABA-CONJUGATES WITH ARACHIDONIC ACID AND PROSTAGLANDIN E₂

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Impairment of cerebral circulation remains one of the main causes of mortality and primary disability of the population all around the world [20], thus representing an important medical and social problem. At the same time, most of the damages after acute and chronic impairment of cerebral blood flow are mediated by excessive formation of free radicals of oxygen and nitrogen, as well as related reactive compounds formed as a result of oxidative stress, which, in turn, leads to the damage of mitochondria and cell membranes. It is well known that global and focal impairment of cerebral perfusion activate a complex cascade of metabolic reactions. Each of these reactions by itself aggravates the damage of nervous system [10].

It is known that the lysosomes of some cells, especially neuronal cells, accumulate net-products of damage, the excess of which can cause irreversible damage in these cells. One such product is lipofuscin, the so-called “ageing pigment”, which contains peroxidized proteins and lipids, the excess of which can lead to the inability of cells to eliminate the products of oxidative damage. It has been experimentally shown that the mechanisms of formation and composition of lysosomal pigments formed during aging and ischemia are very similar [9, 18]. It was also established that focal permanent ischemia caused by occlusion of the middle cerebral artery (MCAO) leads to increase in lipofuscin concentration in both hemispheres of cerebrum [1].

Our previous studies have shown that preparations with GABAergic activity - mexidol and 2-ethyl-6-methyl-3-hydroxypyridine hemisuccinate, prevent excessive accumulation of lipofuscin in brain tissue after focal permanent ischemia caused by MCAO in both intact and damaged hemispheres of the brain [12].

A number of compounds with GABAergic activity, namely, 5-hydroxyadamantane-2-on (5-HO-Ad-2-on) and the GABA-conjugates with arachidonic acid (AA-GABA) and prostaglandin E₂ (PgE₂-GABA), demonstrated cerebrovascular activity in models of global transient cerebral ischemia, combined vascular pathology of the brain and heart, as well as anxiolytic and neuroprotective activity in an experimental model of focal permanent cerebral ischemia [7, 8, 11, 13, 14, 15, 16]. The above data made it important to study the effect of these compounds on the level of lipofuscin in the brain tissue in an experimental model of focal permanent ischemia.

Thus, the aim of the study was to evaluate the effect of 5-HO-Ad-2-on, AA-GABA and PgE₂-GABA, which has shown cerebrovascular, anxiolytic and neuroprotective activity in experimental models of cerebral ischemia, on the levels of lipofuscin – potential marker of cerebral ischemia, in the condition of permanent focal brain ischemia.

Materials and methods

AA-GABA and PgE₂-GABA were synthesized in the Laboratory of Oxylipins of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences [2, 3, 4].

The experiments were done on 90 white rats weighing 150-250 g. The experiments were carried out in compliance with the ethical rules of animal welfare according to the European Communities Council Directive (86/609/EEC).

Model of focal cerebral ischemia was performed by left-sided occlusion of the middle cerebral artery (MCAO) according to A.Tamura et al. [17], in the modification of A.V. Topchyan et al. [19]. The surgery was performed under chloral hydrate anesthesia (400 mg/kg, i.p.). The
quantitative measurement of lipofuscin in the brain tissue was carried out by fluorescence spectroscopy, based on the ability of lipofuscin to fluoresce in wavelength area of 420-470 nm, with an excitation wavelength of 340-370 nm. 0.2 g of brain tissue was homogenized from each hemisphere at a rate of 1300 rpm in a solution of chloroform: methanol (2:1 v/v) in a ratio of 20:1 (v/w). Homogenization was carried out for 1 minute, at room temperature. Subsequently, after extraction of the water-soluble components with an equal volume of distilled water, centrifugation on the T30 apparatus at a speed of 3000 rpm for 1-2 minutes, and separation of the chloroform layer, methanol was added (0.1 ml per each ml of the final solution) to recover the transparency. The resulting solution was exposed to intensive ultraviolet light for 3 minutes to eliminate fat-soluble components that could interfere with measurement (retinol, in particular) [6]. The fluorescence intensity (FI) of chloroform extracts obtained from homogenized brain tissue was measured on a fluorescent spectrophotometer (MF-2A, Hitachi, Ltd. Tokyo, Japan) at an excitation wavelength of 365 nm and emission wave length of 470 nm. Immediately before the measurement, the fluorimeter was calibrated with a solution of quinine sulfate 0.1 μg/ml (CP) in 0.1 N sulfuric acid solution (FI = 60-90) [5].

In the first series of experiments concentration of lipofuscin was measured in both hemispheres in intact animals (n = 10), in the second and third series of experiments in rats under focal permanent cerebral ischemia (n = 10 in each series) after 6th and 12th days of MCAO, and in the last six series in rats receiving treatment with investigational compounds (n = 20 for each compound) on 6th and 12th days of MCAO. Compounds were administered intraperitoneally, once a day: AA-GABA and PgE2-GABA at a dose of 2 mg/kg, 5-HO-Ad-2-on at a dose of 100 mg/kg. The data of the first series were taken as control for the remaining six series. The statistical processing of data was done by Microsoft Office Excel 2010, using the one-way ANOVA variance analysis, with the Student’s T-test.

Results and discussion

The results of the first series of experiments have shown that the concentration of lipofuscin in the left and right hemispheres of intact animals differs insignificantly, (2.5±0.70) FI and (2.6±0.58) FI, respectively (Pic. 1).

The results of the second and third series of experiments revealed statistically significant increase in lipofuscin levels was on the sixth and the twelfth days after MCAO, in both ipsilateral (7.01±1.32) FI, p<0.01) and contralateral (6.62±1.25) FI, p<0.01) hemispheres in comparison with intact animals. Similar, but less significant changes (in the left hemisphere: (5.70±1.34) FI, p<0.01; in the right hemisphere: (5.61±1.14) FI, p<0.01) were observed on the twelfth day after occlusion (Pic. 1).

Moreover, in the left hemisphere, both on the sixth and the twelfth day of ischemia, there is more significant increase in lipofuscin levels in comparison with the right hemisphere. Some observable decrease in lipofuscin levels, which occurs without any medication on 12th day after occlusion, may be a result of intrinsic regenerative processes in the brain tissue.

5-HO-Ad-2-on. Quantitative measurement of lipofuscin after daily intraperitoneal administration of the adamantane derivative (8th and 9th series of experiments) revealed a faster (already after six-day course) decrease in pigment concentration compared with previous two compounds, however, the continuation of treatment within 12 days resulted only an insignificant decrease in lipofuscin concentration in both hemispheres. Thus, the results of the sixth day were (4.01±0.76) FI, p<0.01 and (3.65±0.92) FI, p<0.01, the twelfth day – (3.55±0.64) FI, p<0.01 and (3.26±0.54) FI, p<0.01 for the left and right hemispheres, respectively (Pic. 2).

AA-GABA. Intraperitoneal administration of AA-GABA for six days after MCAO (4th series of experiments) results in a statistically significant decrease in the concentration of lipofuscin, both in the injured-left and in the intact - right hemisphere ((4.51±1.12) FI, p<0.01; (3.94±1.23) FI, p<0.01, respectively), compared with untreated animals.

The administration of the compound for 12 days (5th series of experiments) also results in a statistically significant decrease in lipofuscin content both in the left ((2.61±0.66) FI, p<0.01) and in the right hemisphere ((2.25±0.45) FI, p<0.01), compared with control animals. Moreover, the amount of lipofuscin in the left hemisphere after twelve-day administration of the compound is approaching, and in the right hemisphere it decreases even lower than that of intact rats (Pic. 3).

PgE2-GABA. 6-day treatment with the compound (6th series of experiments) leads to a statistically significant decrease in the amount of lipofuscin in both hemispheres (left hemisphere – (4.36±1.24) FI, p<0.01; right hemisphere – (4.24±1.23) FI, p<0.01).

As for the results of the 7th series of experiments, on the 12 day (left – (3.48±0.97) FI, p<0.01; right –(3.28±0.91)
Fl, p<0.01), the changes are obvious compared with control, but they do not significantly differ from the results of the sixth day, and are not even close to the results of intact animals, which was seen in AA-GABA (Pic. 4).

**Conclusion**

The study results have shown that all three investigated compounds contribute to the prevention of excessive lipofuscin accumulation in both hemispheres of rat brain under the conditions of focal permanent cerebral ischemia by occlusion of left middle cerebral artery. 6-day as well as 12-day intraperitoneal administration of AA-GABA (2 mg/kg) leads to statistically significant prevention of accumulation of lipofuscin in both hemispheres of rat brain with MCAO. Although in comparison with other compounds this effect is weaker on the 6 th day of the treatment, lipofuscin levels after 12-day treatment of this compound are close to the results of intact rats.

The highest rate of prevention of lipofuscin accumulation was observed after a 6-day treatment by 5-HO-Ad-2-on (100 mg/kg), but a 12-day treatment by this compound cedes the effects of analogical duration of treatment by AA-GABA.

A 6-day treatment by PgE 2 -GABA (2 mg/kg) prevents excessive accumulation of lipofuscin in both hemispheres, but a 12-day treatment by this compound also cedes the effects of analogical duration of treatment by AA-GABA.

The obtained data showed that the studied compounds prevent excessive accumulation of the lipofuscin pigment, one of the potential markers of cerebral ischemia, in brain tissues after occlusion of the left middle cerebral artery (both in the ipsilateral and contralateral hemispheres). Based on the previous data, all the studied compounds selectively increase blood supply to the brain after ischemia and also demonstrate GABA A -ergic activity. Issuing from this, it can be assumed that one of the possible mechanisms for preventing the accumulation of lipofuscin in brain tissue is their GABA A -activating effect.

Thus, compounds with selective cerebrovascular and anti-ischemic activity prevent an increase in the content of lipofuscin in the brain tissue caused by ischemic injury. The effect on the quantitative content of lipofuscin demonstrated during MCAO-caused focal permanent ischemia confirms the anti-ischemic activity of the studied compounds.
REFERENCES


REZÜMЕ

КОЛИЧЕСТВЕННЫЕ ИЗМЕНЕНИЯ ЛИПОФУСЦИНА В УСЛОВИЯХ ФОКАЛЬНОЙ ПЕРМАНЕНТНОЙ ИШЕМИИ МОЗГА ПОСЛЕ ВВЕДЕНИЯ 5-ГИДРОКСИАДАМАНТАНА-2-ОН И КОНЬЮГАТОВ ГАМК С АРАХИДОНОВОЙ КИСЛОТОЙ И ПРОСТАГЛАНДИНОМ Е2.

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Ключевые слова: фокальная пермансная ишемия, крысиный мозг, 5-гидроксикарвон, конъюгаты ГАМК с арахидоновой кислотой и простагландин Е2.

Нарушения церебрального кровообращения активируют сложный каскад метаболических реакций, каждый из которых сам по себе усугубляет поражение нервной системы. Одним из конечных продуктов, избыток которых может вызвать в нейронных клетках необратимые повреждения, является липофусцин, так называемый «пигмент старения».

Показано, что механизмы образования и состав лизосомальных пигментов, формирующихся при старении и ишемии, очень схожи. Также установлено, что фокальная пермансная ишемия, вызванная окклюзией средней мозговой артерии, приводит к повышению концентрации липофусцина в обоих полушариях головного мозга.

Целью исследования явилось изучение влияния ряда соединений, проявлявшихся в цереброваскулярной и нейропротекторной активности, а именно: 5-гидроксикарвон, конъюгаты ГАМК с арахидоновой кислотой и простагландин Е2, на уровень липофусцина в тканях головного мозга на модели окклюзии средней мозговой артерии. Результаты исследования показали, что данные соединения предотвращают избыточное накопление липофусцина - потенциальный маркер церебральной ишемии, в тканях головного мозга как в интактных, так и в поврежденных полушариях головного мозга. Одним из возможных механизмов предотвращения накопления липофусцина в тканях головного мозга может быть ГАМК-активирующее действие исследуемых соединений. Продемонстрированное влияние на количество содержание липофусцина в условиях фокальной пермансной ишемии подтверждает противопоезжающую активность исследуемых соединений.