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Influence of 5-hydroxyadamantane-2-on on behavioral changes of rats under conditions of local cerebral ischemia

V. S. Meliksetyan

YSMU, Department of Clinical Pharmacology
2 Koryun str., Yerevan 0025, Armenia

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Stroke is one of the leading causes of death and sustained disability worldwide, and particularly in Armenia. Moreover, the incidence of ischemic stroke events significantly prevails over all types of cerebral blood flow disturbances [6, 11]. Thus, the investigation of drugs improving the condition of ischemic cerebral tissue is reasonable and requires searching of new active chemical entities and new mechanisms of action. One of the approaches to reduce the risk of post-stroke complications is the use of neuroprotective agents capable of acting on pathophysiological mechanisms, thereby enhancing local blood flow and preventing further expansion of ischemic zone. In these terms, compounds that act on GABA system are of high interest, because the role of GABA-dependent mechanisms is crucial. There are literature data stating that compounds, increasing GABA-ergic transmission, have neuroprotective activity [8].

Our attention has been paid to 5-HO-Ad-2-on, an adamantane derivative, the mechanism of action of which and the effects on the cerebral blood flow were studied earlier. Particularly, it has been shown that 5-HO-Ad-2-on (100 mg/kg, i.v.) enhances the local blood flow in the cerebral cortex of rats under the conditions of global transient brain ischemia, while not influencing the brain blood flow in intact rats. In the same dose, adamantane derivative significantly decreases mortality in rats under conditions of hypergravity ischemia. The cerebrovascular effect of 5-HO-Ad-2-on is abolished by bicuculline (GABA-A receptor blocker), which is evidence for a GABA-ergic component in the mechanism of its cerebrovascular action [4]. Under the conditions of permanent OMCA, 5-HO-Ad-2-on recovered compensatory regeneration in neural cells, axons, and glial cells, and the number of microcirculatory vessels was increased [5].

In the course of current study the anxiolytic-like effect, as well as the

effect on rats' memory and learning processes have been demonstrated under the conditions of local cerebral ischemia.

These effects are compared with those of Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate) – a clinically effective neuroprotective drug, having similar mechanisms of action as 5-HO-Ad-2-on [2].

Materials and Methods

A total of 58 adult male white rats, weighting 180-250 g, were used in current study. All animals were housed in standard laboratory conditions of vivarium of YSMU, i. e. 40-70 % relative humidity, 25±2ºC room temperature, 12 h light :12 h dark cycle. Animals were kept in standard laboratory cages, up to 6 rats per cage and were given food and water ad libitum. All experiments were carried out between 9:00 and 14:00.

To evaluate locomotor activity of rats and distinguish them by their phenotypes, "open field" test was used preliminary to the main behavioral test. Only the animals with "middle-activity" were used in further tests, having "general locomotor activity" level between 10 and 30 [3]. As a model of local cerebral ischemia, OMCA was chosen [9]. To prepare for the surgical procedure, animals were narcotized by chloral hydrate solution (400 mg/kg, i/p).

To evaluate animals' behavior, "elevated plus-maze" (EPM) test was implemented. This test reveals the changes in animals' motor activity as well as anxiety-related behavioral changes [7]. The following criteria were measured and used for the statistic calculations: the time spent on open arms (OT), the time spent in the center (CT), the total number of open and closed arm entries (TN) and the percent of open arm entries (ON/TN) [10].

As a test to estimate animals' memory and learning processes, a step-through, one-trial passive avoidance task (PAT) was used [1]. All selected animals were exposed to the formation of avoidance reflex, and the latency period (LP) of this first entrance was registered for each. On the next day, rats were checked for reflex formation: only those with latency period above 300 seconds (thus, with successfully formed avoidance reflex) were selected for the further experiments of memory and learning. Animals that didn't enter the chamber for more than 300 seconds were placed back into the cage, and LP was considered for them equal to 300 seconds.

Animals were divided into 3 groups. The first was the control group (n=26) undergone OMCA and received normal saline solution once daily (10 ml/kg, i/p). Before the OMCA, all animals of this group underwent testing with EPM, as well as were checked by PAT. The animals of the second group (n=14) received Mexidol 30 minutes after OMCA and all the following days, once daily (200 mg/kg, i/p). And finally, animals of the third group (n=18) were treated by 5-HO-Ad-2-on (100 mg/kg, i/p), by the same dosing regimen as Mexidol. All animals were tested by EPM and PAT a day before OMCA and on

the following 6th and 12th days after OMCA, as according to literature data the changes in neurological tissue of rats are mostly profound on these days [9].

The obtained data are represented as mean values with standard deviation (M±SD). Student's t-Test was used to evaluate significance level of differences between groups (Microsoft Excel 2007, 2-tailed, 3-type TTEST). Differences were considered statistically significant when $0.01<p<0.05$ and $p<0.01$.

Results and Discussion

Elevated plus-maze

The results of the first group (Table 1) show that the OMCA leads to all four EPM criteria to decrease compared with intact rats. It can be explained by the development of anxiety-like behavior in rats as a result of cerebral ischemia and conform to available literature data [9]. As calculations show, the vast majority of changes in the tested criteria are statistically significant for both the 6th and the 12th days of OMCA. Moreover, it is worth to mention that three criteria (CT, TN and ON/TN) display some increase on the 12th post-ischemic day, compared with the 6th day, nevertheless being statistically lower than those of intact group. This can be explained by the involvement of compensatory protective mechanisms against ischemia.

Table 1

Mean results of EPM testing

Group	Day	OT (c) M ± SD	CT (c) M ± SD	TN M ± SD	ON/TN (%) M ± SD
Intact	0 day (n=26)	59.77 ± 59.24	60.62 ± 34.67	4.08 ± 1.92	42.18 ± 27.02
Saline	6th day (n=13)	13.54 ± 20.61 (↓4.41x) p_0^*	13.77 ± 10.54 (↓4.4x) p_0^*	1.92 ± 0.76 (↓2.13x) p_0^*	23.08 ± 23.11 (↓1.83x) p_0^{**}
	12th day (n=11)	11.55 ± 14.39 (↓5.18x) p_0^*	18.27 ± 14.64 (↓3.32x) p_0^*	2.09 ± 0.94 (↓1.95x) p_0^*	24.24 ± 23.99 (↓1.74x)
Mexidol	6th day (n=14)	69.64 ± 58.91 (↑5.14x) p_1^*	51.21 ± 33.89 (↑3.72x) p_1^*	3.29 ± 1.59 (↑1.71x) p_1^*	44.05 ± 21.65 (↑1.91x) p_1^{**}
	12th day (n=11)	77.27 ± 68.97 (↑6.69x) p_1^{**}	96.82 ± 56.24 (↑5.3x) p_1^*	2.91 ± 1.38 (↑1.39x)	46.21 ± 30.13 (↑1.91x)
5-HO-Ad-2-on	6th day (n=18)	80.00 ± 66.92 (↑5.91x) p_1^*	61.17 ± 40.72 (↑4.44x) p_1^*	3.39 ± 1.85 (↑1.77x) p_1^*	48.45 ± 35.21 (↑2.1x) p_1^{**}
	12th day (n=13)	72.00 ± 69.73 (↑6.23x) p_1^*	50.46 ± 36.27 (↑2.76x) p_1^*	3.77 ± 2.35 (↑1.8x) p_1^{**}	44.2 ± 35.49 (↑1.82x)

OT – time spent on open arms, CT – time spent in the center, TN – total number of open and closed arm entries, ON/TN – percent of open arm entries

↓ – decreased compared with control; ↑ – increased compared with control; * – $p<0.01$,

** – $0.01<p<0.05$ (p_0 – compared with intact group, p_1 – compared with Saline group).

The results of the second and third groups, receiving Mexidol and 5-HO-Ad-2-on respectively, are represented for each EPM parameter separately.

As we can see on the Fig. 1, both Mexidol and 5-HO-Ad-2-on display statistically significant rise in OT parameter, compared with saline-treated groups (Table 1). Moreover, the results of the 6th day (Mexidol - 5.14 times, $p<0.01$; 5-HO-Ad-2-on – 5.91 times, $p<0.01$) are slightly lower from those of the 12th day (Mexidol – 6.69 times, $0.01<p<0.05$; 5-HO-Ad-2-on – 6.23 times, $p<0.01$).

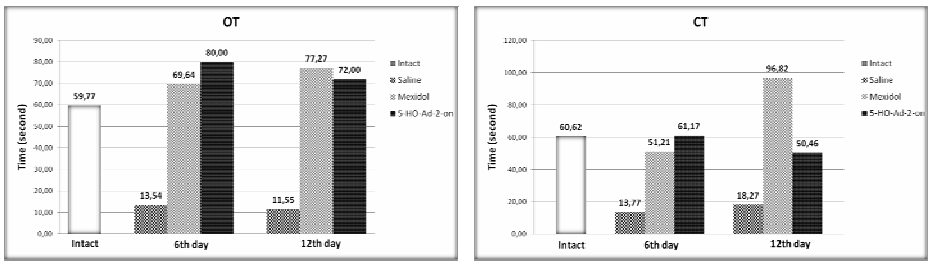

Fig. 1 and 2. The comparison of OT and CT between treatments by Mexidol, 5-HO-Ad-2-on, normal saline solution (Saline) after OMCA, and intact rats.

CT was the second EPM parameter to be evaluated (Table 1, Fig. 2). Here the improvement is less than for the previous parameter, but also statistically significant. Although 5-HO-Ad-2-on reaches higher values of CT on the 6th day (61.17 ± 40.72), than Mexidol (51.21 ± 33.89), the latter exceeds its own value of the 6th day almost twice on the 12th day of treatment (96.82 ± 56.24), as well as value of 5-HO-Ad-2-on of the same day (50.46 ± 36.27).

The results of TN parameter (Table 1, Fig. 3) reveal higher improving tendency in the group, treated by 5-HO-Ad-2-on, especially on the 12th day (6th day - 1.77 times, $p<0.01$ and 12th day – 1.8 times, $0.01<p<0.05$).

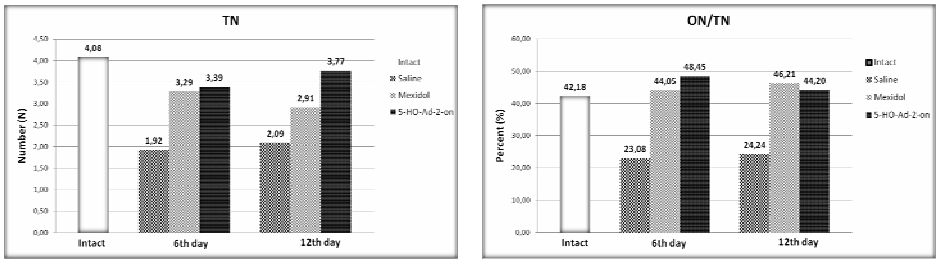

Fig. 3 and 4. The comparison of TN and ON/TN between treatments by Mexidol, 5-HO-Ad-2-on, normal saline solution (Saline) after OMCA, and intact rats.

Finally, the dynamics of change of the parameter ON/TN is very close to the first parameter (OT). Here the difference between the results of the 6th and 12th days of treatment, as well as between two treatment groups, is negligible.

Nevertheless, the improvement compared to the saline-treated group is obvious for both treatment groups (Table 1, Fig. 4).

Thus, the results of EPM show that both Mexidol and 5-HO-Ad-2-on increase the parameters of EPM, hence indicating their capability to prevent the formation of anxiety-like behavior in rats after the OMCA. Moreover, the results of both the 6th and 12th days are reaching the values close to the results of intact group.

Passive avoidance task

The results of the first group of passive avoidance task show that the mean latency period is very high (278.15 ± 56.65) and close to the highest possible value (300). This means that the formation of avoidance reflex was successful for the majority of animals. OMCA brings to statistically significant decrease of LP both on the 6th and 12th days: 188.08 ± 129.2, $0.01<p<0.05$ and 163.64 ± 134.02, $0.01<p<0.05$ respectively (Table 2).

Table 2

The values of latency period

	Intact		**OMCA + Saline**		**OMCA + Mexidol**		**OMCA + 5-HO-Ad-2-on**	
Terms	Before r.f. (n=26)	After r.f. (n=26)	6th day (n=13)	12th day (n=11)	6th day (n=14)	12th day (n=11)	6th day (n=18)	12th day (n=13)
LP (c) M±SD	21.65 ± 19.91	278.15 ± 56.65	188.08 ± 129.2##	163.64 ± 134.02##	271.43 ± 61.03**	233.27 ± 115.01	261.11 ± 90.36	263.77 ± 88.48**

- $0.01<p<005$, compared with intact group (after reflex formation)

** - $0.01<p<0.05$, compared with saline-treated group of the same day

The results of the second group revealed statistically significant increase in LP, compared with saline-treated group on the 6th day (271.43 ± 61.03; $0.01<p<0.05$), although the increase of 12th day was not statistically significant (233.27 ± 115.01) (Table 2).

Finally, the mean LP values for the third group, receiving 5-HO-Ad-2-on, are as follows: 261.11 ± 90.36 and 263.77 ± 88.48 ($0.01<p<0.05$) for the 6th and 12th days respectively (Table 2). As the results show, both on the 6th and 12th days the improvement of memory and learning is considerable and the values of LP are very close to the value of intact group.

Following the results of PAT testing, it is worth to mention that 5-HO-Ad-2-on considerably prevents the lack of memory and learning processes in a similar way as Mexidol on the 6th day, considerably exceeding Mexidol on the 12th day of the treatment.

Summing up the results of both behavioral tests, it can be concluded that 5-hydroxyadamantane-2-on significantly prevents some behavioral outcomes of

ischemic stroke caused by OMCA. In particular, the parameters indicating anxiety as well as disturbances of memory and learning process are shown to be improved. Moreover, some positive changes of behavioral outcomes even exceeded the results of Mexidol, particularly latent period of PAT as well as total number of entries of EPM-test for the 12th day of OMCA.

The obtained data could serve as a basis for the further investigation of 5-hydroxyadamantane-2-on as a potential effective agent for the management of stroke and its complications.

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Влияние 5-гидроксиадамантан-2-она на поведенческие изменения крыс в условиях локальной ишемии мозга

В. С. Меликсетян

В ходе исследования было изучено анксиолитическое действие 5-гидроксиадамантан-2-она, а также его способность предотвращать ухудшение памяти и процессов обучения крыс, перенесших окклюзию левой средней мозговой артерии. Для оценки тревожности были выбраны следующие параметры теста “приподнятый крестообразный лабиринт”: время, проведенное в открытых рукавах, время, проведенное в центре, общее количество заходов в открытые и закрытые рукава и процент заходов в открытые рукава. Процесс обучения и формирования памяти у животных был оценен с применением “условного рефлекса пассивного избегания” и вычислением латентного периода. Упомянутые эффекты исследуемого соединения были сравнены с контрольной группой, получавшей изотонический раствор натрия хлорида, а также с широко применяемым в клинике препаратом мексидол.

Было показано, что ежедневное интраперитонеальное введение 5-гидроксиадамантан-2-она в дозе 100 мг/кг способствует устранению тревожности животных, перенесших окклюзию левой средней мозговой артерии, а также предотвращает ухудшение памяти и процессов обучения. Было проведено сравнение эффектов на 6-й и 12-й день после перевязки.

5-հիդրօքսիադամանտան-2-օնի ազդեցությունն առնետների վարքագծային փոփոխությունների վրա ուղեղի լոկալ իշեմիայի պայմաններում

Վ. Ս. Մելիքսեթյան

Հետազոտության սահմաններում ուսումնասիրվել է 5-հիդրօքսիադամանտան-2-օն միացության տագնապամարիչ ազդեցությունը,

ինչպես նաև վերջինիս ազդեցությունն առնետների հիշողության և ուսուցման գործընթացների վրա: Նշված վարքագծային խանգարումների փորձարարական մոդելավորման նպատակով կատարվել է առնետների ձախ միջին ուղեղային զարկերակի կապում՝ վերջիններիս մոտ սուր իշեմիկ կաթվածի առաջացումով: Կենդանիների տագնապային վարքը գնահատելու նպատակով հաշվարկվել են «բարձրացված խաչաձև լաբիրինթոս» թեստի չորս հիմնական չափանիշները՝ բաց թևերում անցկացրած ժամանակը, կենտրոնում անցկացրած ժամանակը, բաց և փակ թևերի մուտքերի ընդհանուր քանակը և բաց թևերի մուտքերի տոկոսը: Հիշողության և ուսուցման գործընթացի հետազոտությունը կատարվել է «պասիվ խուսափման պայմանական ռեֆլեքսի» կիրառմամբ՝ հաշվարկելով մուտքի լատենտ ժամանակաշրջանը: Հետազոտվող միացության նշված հատկությունները համեմատվել են նյարդաբանական պրակտիկայում կիրառվող մեքսիդոլ դեղի հետ, իսկ որպես ստուգիչ խումբ ընտրվել են առնենտներ, որոնք միջին ուղեղային զարկերակի կապումից հետո դեղանյութի փոխարեն ստացել են նատրիումի քլորիդի իզոտոնիկ լուծույթ: Կատարվել է վիրահատությունից հետո 6-րդ և 12-րդ օրվա արդյունքների համեմատություն:

Հետազոտության արդյունքները վկայում են, որ 5-հիդրոքսիադամանտան-2-օնը 100 մգ/կգ դեղաչափով (ներորովայնային, օրը մեկ անգամ) կանխում է առնետների մոտ ուղեղի լոկալ իշեմիայի արդյունքում դրսևորվող տագնապի զարգացումը, ինչպես նաև հիշողության և ուսուցման գործընթացների խանգարումը:

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