The Effects of Idazoxan and Efaroxan Improves Memory and Cognitive Functions in Rats

Experimental research

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Investigating the effects of idazoxan and efaroxan imidazoline receptor antagonists on cognitive functions with the rat Y-maze test; an internationally recognized experimental pattern of behavior, is to be used in order to evaluate the effects of test substances on the simple spatial memory of the laboratory animals. Our experimental evaluation tested the influence induced by idazoxan and efaroxan on the short-term memory on rats. In the experiment were used eighteen (18) male Wistar rats which were randomly divided into three groups (I - Control, II - IDZ and III - EFR) comprising of 6 animals each, treated intraperitoneally according to the following protocol: group I (Control): distilled water 0.5 mL/100 g body weight; group II (IDZ): idazoxan 3 mg/kg body weight; group III (EFR): efaroxan 1 mg/kg body weight. The purpose of this research was to assess the eligibility using the Y-maze test, involving: latency of the first arm visited, the number of arms visited, and the time spent into the arms, the number of returns of the experimental animals in the same arm, the number of alternations, percentage of spontaneous alternation. In this work, manifestations of the natural behavior of the animals tested was expressed by their choice of goal arm alternation. Statistical data processing reveals that: the administration of IDZ, as well as of EFR was accompanied by a tendency to enter a less recently visited arm and reduced the total number of arms visited, statistically significant ($p<0.05$) compared to control group. In this experiment, the use of these two imidazoline receptor antagonists did not considerably influence the reference memory, when pursuing the latency of the first arm visited, compared to the group treated with distilled water. The effects of IDZ and EFR on the percentage of time spent in the arms were sorted in descending order in this behavioral experimental model (IDZ>Control>EFR). The results demonstrate that the treatment with imidazoline agents optimizes the cognitive function of the animals, improving their learning ability, in the rat Y-maze Test. Regular exercise can reduce depression-, anxiety-, and impaired cognitive-like behaviors, and in conclusion these substances would be a useful pharmacological agents for the treatment of cognitive dysfunction.

Keywords: imidazoline (I) receptor antagonists, idazoxan, efaroxan, memory

To reconcile distribution and pharmacological data, imidazoline (I) receptors have been involved in the regulation of the blood pressure, anti-inflammation, hyperglycemia, antitumor activities and cognitive function. Studying their distribution in the central nervous system plays an important role in the formation of long-term memories and therefore establish a neurological basis for the complex pharmacological effects of centrally acting idazoxan and efaroxan. Imidazoline receptors constitute a family of non-adrenergic high-affinity binding sites for clonidine, idazoxan and allied drugs [1].

Imidazoline system is one of the major structures involved in the functioning of the human brain, but on the other hand I_2 receptors have been found in the liver, platelets, adipocytes, kidneys, adrenal medulla and brain, including the frontal cortex. The imidazoline receptor protein has a molecular mass of 70 kDa, but the exact amino acid sequence is not yet known. Imidazoline receptor identification and discovery of substances with agonistic or antagonistic activity paved the way for complex researches, experimental and clinical studies, to clarify their involvement in the pathophysiological mechanisms of multiple diseases [2]. Literature data revealed that, the imidazoline receptors located on both centrally and in the peripheral sites are involved in numerous patho-physiological processes, however, the mechanisms underlying them are not fully deciphered. The evidence acquired until now suggests that I_2 receptors have an antinociceptive effect[3]. A complex relationship exists between Agmatine and the body's response to stress, such as: in analgesia, drug addiction, withdrawal syndrome and in neuroprotection [4,5]. Therefore, studies had investigated the role of agmatine in ethanol-induced anxiolysis and withdrawal anxiety using elevated plus maze. Alcohol motivation can be stopped by agmatine ability to alleviate stress, including stress associated with periods of abstinence following chronic consumption. These results suggest that agmatine and the imidazoline receptor system may be implicated in overcoming alcohol withdrawal symptoms such as anxiety. Imidazoline receptors may play an important role in the treatment of concomitant alcohol and tobacco consumption, but also influence central antinociceptive activity. It has been discovered that efaroxane and idazoxane have been ineffectually antagonizing ethanol or nicotine-induced antinociceptosis. Endogenous imidazoline receptors ligands, harmane and agmatine as well as the imidazolin l_1 (á2 adrenergic receptor agonist, clonidine, I_1 agonist moxonidine and imidazoline I_2 agonist, 2-BFI have increased the antinociceptive effects of ethanol and nicotine [6,7].

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There are further investigations of related studies that indicated the role of substances acting on the imidazoline receptors, on behavior, remembrance, eagerness to learn, locomotor activity and sedation. Finally, progress will also depend on the means of understanding the structural and functional organization of the receptors in the rat cerebral cortex from an interdisciplinary perspective, including psychiatry, psychology, behavioral sciences, genetics, and other neurosciences [8-10]. Idazoxan and Efaroxan have a certain potential for being used in new drug development based on the results that have been reported after conducting numerous behavioral animal models, but their clinical use is not implemented yet.

Based on these findings, efaroxan and idazoxan blocked the anti-compulsive effects of agmatine, suggesting a beneficial effect in stress-related disorders, such as depression, anxiety and posttraumatic stress disorder. For instance, agmatine is an endogenous neuromodulator of mental stress as the results reported agmatine injections into rats and mice also elicit acute anxiolytic-like behavioral changes in the elevated plus maze stress test [11,12]. It has also been observed that activation of imidazoline I_2a in the adrenal glands, by Agmatine, reduces the plasma glucose level in rats using Streptozotocin - induced experimental diabetes [5,13].

In most cases, studying the imidazoline agents on motor behavior and spatial memory justifies the present experimental researches, trying to elucidate the mechanisms that are not fully understood, of both imidazoline receptor antagonists idazoxan and efaroxan, and to assess their involvement in mediating stress and cognitive functions in laboratory animals [14]. The aim of the paper is to evaluate through Y-maze test the effects of two imidazoline receptor antagonists idazoxan [2-(2,3-Dihydro-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazol] and efaroxan [2-(2-Ethyl-2,3-dihydro-1-benzofuran-2-yl)-4,5-dihydro-1H-imidazol] regarding some aspects of memory in rodents.

Experimental part

Material and method

All experiments were approved by our University's Committee for Research and Ethical Issues in compliance with the ethical regulations of the European Community regarding the handling of laboratory animals [15].

Animals and experimental protocol

The research was performed on white male Wistar rats (150–200g), randomly assigned into 3 groups of 6 animals each: group I (Control): distilled water 0.5 mL/100 g body weight; group II (IDZ): 3 mg/kg body weight idazoxan; group III (EFR): 1 mg/kg body weight efaroxan.

On the day of the experiment, the animals were allowed to accommodate in the test room for approximately one hour, after which the test substances were appropriately administered. After injecting the substances, the animals were allowed to rest for 15 minutes, after which they were subjected to the Y-maze test at intervals of 8 minutes.

Rats were kept in special cages in standard laboratory conditions with 23±1°C environment temperature, relative humidity 55-65%, and 12 h artificial light/dark cycle. Water and standardized granulated food were provided *ad libidum*, except for the periods of the studies. Two hours before the investigations, the animals were positioned on a raised wire mesh, under a transparent plexiglass box, in order to familiarize with the testing location. Each rat was used once only, and the interval of the investigation was set as short as possible, to minimize the animal's suffering. Due to ethical considerations, all the animals were euthanized at the end of testing.

The imidazoline (I) receptor antagonists, idazoxan and efaroxan, purchased from Sigma-Aldrich Chemical Company, Germany, were dissolved in distilled water, the solutions being prepared extemporaneously. This test is a classic behavioral model based on the spontaneous tendency of the animal to explore a new environment, in which no food rewards are used, but only the natural behavior of the animal to recognize the space in which it was introduced.

The implication of imidazoline agents in maintaining spatial knowledge, the labyrinth test, was used to highlight the performance of rat work memory. (Y-maze, T-maze, elevated arm maze, swimming test) [16,17]. This experimental model is based on the use of a Y-shaped device with three identical arms (40 x 9 x 16 cm) at 120 ° to each other and a triangular central platform. Each arm has different design elements on the inside of the walls to allow animals to differentiate one arm from another.

This apparatus consists on the distal part of each arm, a detachable recessed cup that can be placed or removed by being covered up. The scheme of this maze ensures that the rat, before starting a new exploration, remembers the arm it has just explored. The animal always has two potential choices during an eight minutes session.

A video camera placed above the device and connected to a computer in another room, was used to evaluate the animal's spontaneous alternation. Data for each measure from the sessions of testing has the following objectives: latency to leave the start arm, latency of first arm visit, number of arms visited and alternate arm returns.

It is considered an entry into the arm if the animal penetrates with all the paws inside it. Normally, after the animal has left an arm, it recognizes the area that has just been explored and has a natural tendency to enter the third different arm of the device, thereby making full alternation [18].

This experimental model is used to highlight whether the animal recalls the arm that has just been explored and consequently enters the other arm of the device. It is considered an initial behavioral test for assessing the functional memory of the laboratory animal [19]. Revisits of the arms previously entered were scored as an error.

Spontaneous alternation (%) is calculated using the formula = Alternations number / (Total number of visited arms - 2) [19,20].

In this conduct test model, the diminishing in errors of working memory (pertinent components for estimating transient memory) implies a facilitator impact on the memory. Subsequently, the Y maze test was conducted to evaluate the learning and memory abilities of the rats, with the purpose of elucidating the pharmacological mechanisms of idazoxan and efaroxan [21,22].

The decreasing of time relates to an improvement of the discriminative spatial memory by expanding the precision of the arm in Y maze while keeping under observation the cognitive functions and physical condition of the rats [23]. The diminishing in the quantity of reference errors (vital components to evaluate the long-haul memory), compares to a positive impact on the long-term memory [24,25].

The information was exhibited as math mean ± standard deviation (SD) of mean, and investigated utilizing SPSS program for Windows 10, variant 17.0, ANOVA technique. P-values under 0.05 were viewed as measurably noteworthy contrasted with control gathering.

Results and discussions

The centralization of the data revealed that:

- the administration of IDZ (3 mg / kg body weight) did not significantly affect the latency of leaving the home area (3.40 ± 0.35 seconds) compared to the treated group with distilled water (3.12 ± 1.50 seconds) in the rat Y-maze test;

- intraperitoneal injection of EFR (1 mg / kg body weight) did not produce significant changes in latency of leaving the baseline area (3.27 ± 0.21 seconds) compared to the control group (3.12 ± 1.50 seconds) over the same duration in the same test behavior; (fig. 1).

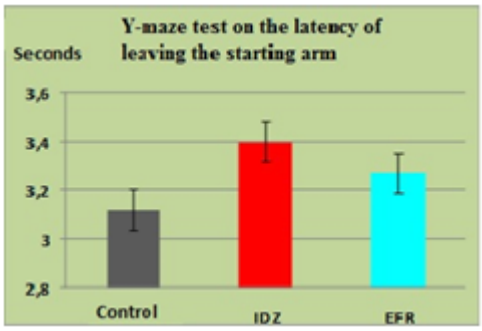

Fig. 1. Effects of IDZ and EFR on the Y-maze test on the latency of leaving the starting arm. Values are expressed as the mean ± standard error of the mean. *p<0.05, **p<0.01

The effects of IDZ and EFR on the latency to leave the start area were in descending order the following: **IDZ > EFR > Control**. The effects of IDZ and EFR on the latency to enter in the first arm were in descending order the following: **EFR > IDZ > Control** (table 1).

Table 1

THE EFFECTS OF IDZ AND EFR ON Y-MAZE TEST -THE LATENCY TO LEAVE THE START AREA. VALUES ARE EXPRESSED AS THE MEAN±S.E.M. *p<0.05, **p<0.01 vs CONTROL.

	The latency to enter in the first arm (s)
Control	20.15±1.21
IDZ	21.33±0.38
EFR	21.67±1.09

Harmonic media for 6 animals was used. The effects of IDZ and EFR on the total number of visited arms were sorted in descending order as follows: **Control > IDZ > EFR** (fig 2).

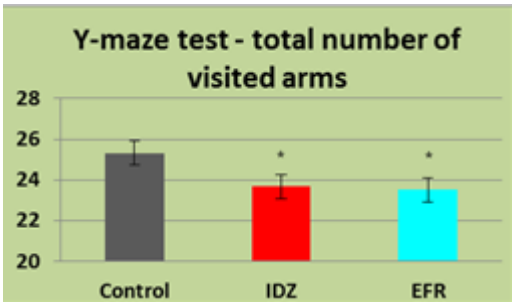

Fig. 2. Effects of IDZ and EFR on the Y-maze test on the total number of arms visited. Values are expressed as the mean ± standard error of the mean. *p<0.05, **p<0.01.

The effects of IDZ and EFR on the number of returns in the same arm were sorted in descending order as follows (fig.3): **Control> EFR> IDZ**

The effects of IDZ and EFR on the percentage of time spent in arms were in descending order the following: **IDZ > Control > EFR** (table 2).

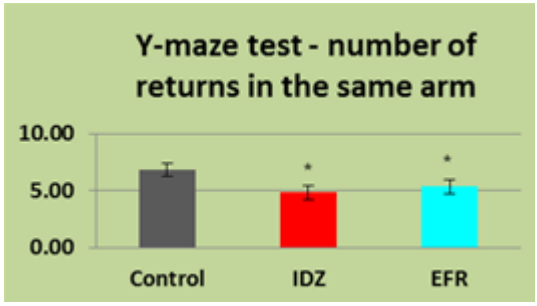

Fig. 3. Effects of IDZ and EFR on the Y-Maze test in terms of the number of backsets within the same arm. Values are expressed as the mean ± standard error of the mean. *p<0.05, **p<0.01

Table 2

THE EFFECTS OF IDZ AND EFR ON Y-MAZE TEST – THE PERCENTAGE OF TIME SPENT IN ARMS. VALUES ARE EXPRESSED AS THE MEAN±S.E.M. *p<0.05, **p<0.01 vs CONTROL

	The percentage of time spent in arms
Control	30.83±1.47
IDZ	31.50±1.05
EFR	30.17±1.33

The effects of IDZ and EFR on the number of alternations and on the spontaneous alternation percentage were in descending order as follows: **IDZ > EFR > Control** (fig 4).

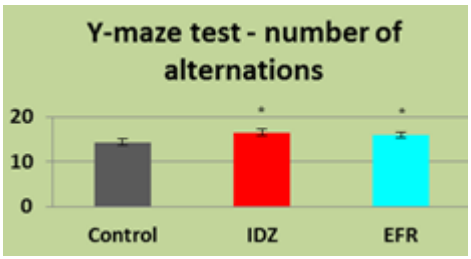

Fig. 4. The effects of IDZ and EFR on Y-maze test - the number of alternations

The centralization of the results revealed that: the use of IDZ (3 mg / kg body) increased the number of alternations (16.50 ± 1.38), statistically significant (* p <0.05), compared to the distilled water lot (14.35 ± 1.22) laboratory animal (fig 5).

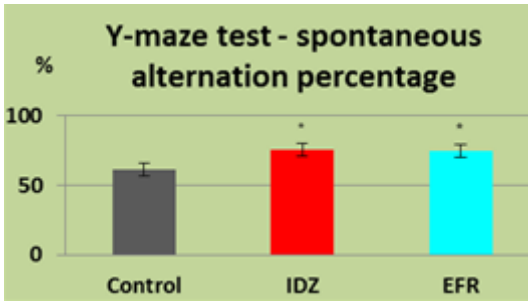

Fig. 5. The effects of IDZ and EFR on Y-maze test - the number of alternations and on the spontaneous alternation percentage. Values are expressed as the mean±S.E.M.*p<0.05, **p<0.01 vs control.

In our research, we investigated the effects of imidazoline antagonists idazoxan and efaroxane on spontaneous rat behavior through the Y-maze test, which is a valuable method for assessing the central neurobehavioral effects of the investigated substances. The Y-maze test is a classic internationally standardized behavioral pattern, constituting an easy screening method to study the effects of various pharmacologically active substances on cognitive functions of animals [25,26].

We obtained information that attests the following aspect: efaroxan showed a stronger effect of reducing the number of arms visited (in correlation to the exploratory activity) compared to idazoxan on this pattern of repetitive behavior. There were no significant changes in the latency of leaving the home area, the latency of the first arm visit of the device, but the percentage of time spent in the arm of the device, between the batches that received idazoxan and efaroxan and the group treated with distilled water, suggests that imidazoline antagonists does not influence motor activity in the Y-maze test.

An experimental study conducted in 2014 demonstrated the involvement of imidazoline receptors in inhibiting rat learning activities [11]. Retrodialysis of the imidazoline I_2-idazoxan receptor antagonist resulted in the potentiation of noradrenaline release induced by basolateral tricuspid shock in the rat [27].

Recent experimental studies revealed neuroprotective effects of imidazoline I_2 receptor involvement focused on the study of two I_1, I_2 imidazoline and alpha2 adrenoreceptor antagonists: efaroxane and idazoxane. Also, some investigations in mice with experimentally induced autoimmune encephalomyelitis have demonstrated improvement in structural changes in the brain and reduction of lesions in the blood-brain barrier [28-30]. Dexafaroxan has been shown to exhibit neuroprotective effects on devascularization-induced neurodegeneration, ameliorate structural changes in the hippocampus and remove cognitive deficits induced by cerebral ischemia in the rat [31], as well as in the excitotoxic lesions produced at the basal magnocellular core region, and increase the olfactory discriminative capacity [32], suggesting its use in the treatment of memory disorders in Alzheimer's disease [33]. In Alzheimer's disease, the appearance of senile and neurofibril plaques is comprehensive, progressive. Other changes include the reduction in the synthesis of acetylcholine or other neurotransmitters (dopamine, noradrenaline, serotonin and their metabolic products).

Deficit and disturbance of acetylcholine function leads to memory disorders, disruption of dopamine function leads to Parkinsonian disorders and disorders of serotonin, noradrenaline function in depressive disorders. When the quantity of neurotransmitters (eg noradrenaline, serotonin, dopamine, epinephrine, norepinephrine, GABA) is not well balanced, the messages cannot be properly transmitted and reactions specific to anxiety and / or depression may occur. Norepinephrine is the hormone that controls the body's response to stress. It is thought that depression is caused by the too low number of serotonin receptors. Experimental research on the effects of idazoxan and efaroxan on some oxidative stress markers in rats subjected to forced exercise in the treadmill test, suggests that idazoxan effects on the motor performances were more accentuated, but the antioxidant effects were of low intensity, compared with those of efaroxan, in forced locomotion test on rats [35].

The investigation of changes produced on the markers of oxidative stress (superoxide dismutase - SOD and glutathione peroxidase - GRX) were carried out using the venous blood (0.5 mL) collected from the retro-orbital plexus of the rat, under anesthesia with enflurane. Researchers in medicine continue to look for more data about the possible biological causes of anxiety.

Clinical trials conducted on volunteers who have been subjected to standardized tests of memory and attention have shown that idazoxan does not significantly affect mood, logical reasoning, retrieval memory, and sustained attention, although it has been found to improve selective attention (the effect of site repetition), suggesting that this imidazolinone antagonist improves selective performance and attention [36].

Other clinical trials reveal that the association of idazoxan with clonidine is potentiated and does not antagonize some of its effects on cognitive functions in volunteers subjected to a series of cognitive specific tests for frontal lobe dysfunction, where the imidazolinone antagonist has not been shown to block the favorable effects of clonidine on sustained visual attention and on improved performance of the session after session evaluation [37].

On experimental models: conditional avoidance, NMDA - induced schizophrenia, D-amphetamine induced locomotor disturbances, association of idazoxan with haloperidol produces beneficial effects on behavioral disorders [38].

Recent experimental studies have shown neuroprotective effects of idazoxan, amelioration of structural changes in the brain and reduction of haematoencephalic barrier lesions in mice with experimentally induced autoimmune encephalomyelitis [39].

Conclusions

The idazoxan effects were more pronounced than those of efaroxan in this behavior model in rats. The administration of idazoxan (3 mg/kbw), respectively of efaroxan (1 mg/kgw) was associated with the facilitation of laboratory animals' short-term memory and the reference memory in Y-maze test.

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