IMIDAZOLINE RECEPTOR ANTAGONISTS IDAZOXAN AND EFAROXAN ENHANCE LOCOMOTOR FUNCTIONS IN RATS

Gabriela Rusu[1], Liliana Mititelu-Tartau[1*], Catalina Elena Lupusoru[1], R.V. Lupusoru[1], Ioana Grigoraş[2], Graţiela Popa[3], M. Nechifor[1]

University of Medicine and Pharmacy "Grigore T. Popa"- Iasi

Faculty of Medicine

1. Department of Morpho-Functional Sciences

2. Department of Surgery

Faculty of Pharmacy

3. Department of Pharmaceutical Technology

*Corresponding author. E-mail: lylytartau@yahoo.com

IMIDAZOLINE RECEPTOR ANTAGONISTS IDAZOXAN AND EFAROXAN ENHANCE LOCOMOTOR FUNCTIONS IN RATS (Abstract) Discovered in 1984, imidazoline receptors (I_1, I_2, I_3) are located centrally and peripherally being involved in various physiologic and pathophysiologic processes in the body. Experimental and clinical investigations have suggested the interrelations between imidazoline, adrenergic, dopaminergic, glutamatergic and opioid systems, which may explain the influence of different substances acting on imidazoline receptors in cognitive disorders, behavioral disturbances and motor diseases pathways. **Aim:** To investigate the effects of two imidazoline receptor antagonists on locomotor activity and endurance capacity in rats. **Material and methods:** The experiment was carried out with white male Wistar rats (200-250g) divided into 3 groups of 7 animals each, treated intraperitonealy with the same volume of solution as follows: Group I (Control): distilled water 0.3ml/100 g body weight; Group II (IDZ): idazoxan 3 mg/kbw; Group III (EFR): efaroxan 1 mg/kbw. Exercise capacity was evaluated using a locomotor PanLAB treadmill test. The data were presented as mean +/- standard deviation and significance was tested by SPSS Statistics for Windows version 17.0 and ANOVA method. Experimental protocol was implemented according to recommendations of the Gr.T. Popa" University Committee for Research and Ethical Issues. **Results:** Intraperitoneal administration of idazoxan and efaroxan resulted in a significant increase in running distance compared with the control group ($p<0.05$). At the same time a reduction in the number and time of electric shocks delivered to motivate the animal to keep running was observed. In this experimental behavioral model the effects of idazoxan on the evaluated parameters were more intense than those of efaroxan. **Conclusions:** In our experimental conditions we demonstrated the ability of imidazoline receptor antagonists idazoxan and efaroxan to improve fatigue resistance during forced running in rats. **Keywords:** IDAZOXAN, EFAROXAN, TREADMILL, LOCOMOTOR.

Imidazoline receptors were identified for the first time in 1984, three types being known today: I_1, I_2 and I_3. I_2 imidazoline receptors have been subdivided into two subtypes according to their affinity for amiloride: I_2A subtype is amiloride-

sensitive and I_2B subtype is amiloride-insensitive (1).

Literature data revealed that imidazoline receptors are located centrally and peripherally, being involved in numerous physiological and pathophysiological processes, but the mechanisms are not yet fully understood (2).

Until now four endogenous imidazoline receptors ligands have been characterized, of which agmatine (decarboxylated arginine) is the most known and studied. The others are: two varieties of beta-carbolines: harmine and harmaline and imidazoleacetic acid robotide (3).

Imidazoline receptors have a great affinity for binding to various compounds with imidazolinic structure. Most of the ligands with affinity for imidazoline receptors also bind to alpha-2 adrenergic receptors, these two types of imidazoline receptors being difficult to differentiate (4). Modern methods, such as autoradiography and radio-ligation techniques, offer only indirect evidence regarding the presence of distinct binding sites for alpha-2 and imidazoline receptors (2).

Numerous experimental studies and clinical trials have showed the involvement of imidazoline receptors in different physiologic and pathophysiologic conditions in the body.

- I_1 imidazoline receptors mediate sympathetic inhibitory actions of imidazoline derivatives associated with the decrease of blood pressure (5);
- I_2 imidazoline receptors modulate the level of central monoamines and activate hypothalamic adrenal pituitary axis with a potential antidepressant activity (6);
- I_3 imidazoline receptors regulate insulin secretion in the beta Langerhans pancreatic cells (7).

The potent neurotransmitter agmatine is involved in the mediation of stress response, analgesia, drug addiction, withdrawal syndrome, and in the modulation of seizures development and in neuroprotection (1, 6).

Various experimental and clinical studies suggested the interrelations between imidazolinic, adrenergic, dopaminergic, glutamatergic and opioid systems; thus they might explain the influence of different substances acting on imidazoline receptors on cognitive disorders, behaviour disturbances and in motor diseases pathways. (8, 9) The results reported in the literature are inconsistent and some of them controversial.

The investigations regarding the involvement of substances acting on imidazoline receptors in behaviour, memory, and locomotor activity might lead to new data on the pathophysiological mechanisms of spontaneous behaviour alterations and cognitive disturbances (6, 9).

Idazoxan is a 2-(2,3-dihydro-1,4-benzodioxine-2-yl)-4,5-dihydro-1H-imidazole derivative that selectively blocks alpha-2 adrenergic and imidazoline receptors. Various studies have been performed to investigate its effects in depression, schizophrenia and different types of psychosis, but the results are not yet validated (10, 11). Some recent researches demonstrated the neuroprotective activity of idazoxan with a reduction of blood brain barrier damage during experimental autoimmune encephalomyelitis in mouse (12).

Efaroxan, 2-(2-Ethyl-2,3-dihydro-1-benzofuran-2-yl)-4,5-dihydro-1H-imidazole derivative is an alpha-2 adrenergic receptor antagonist and also an imidazoline receptor ligand (2, 5). In the last few years investigations on the involvement of this imidazo-

line receptor antagonist in the regulation of blood pressure, pancreatic insulin secretion and also in depressive conditions have been conducted (2, 5, 13).

The aim of this study was to investigate the effects of imidazoline receptor antagonists idazoxan and efaroxan on locomotor activity and endurance capacity in rats.

MATERIAL AND METHODS

The experiment was carried on white Wistar rats (200-250g). Animals were housed in plastic cages in an animal room maintained at 23 ± 1°C on a 12-hour dark cycle (light period, 07:00 – 19:00). Standard laboratory food and tap water were freely available, except during the time of the experiments.

Rats were divided randomly into four groups 7 animals each, treated intraperitoneally with a single dose as follows: Group I (Control): distilled water 0.3 ml/100g weight; Group II (IDZ): idazoxan 3 mg/kbw; Group III (EFR): efaroxan 1 mg/kbw.

Idazoxan and efaroxan (purchased from Sigma Aldrich Chemical Co, Germany) were diluted in distilled water and prepared extemporaneously.

Exercise capacity was assessed using a PanLAB treadmill for locomotor-fatigue. This apparatus consists of a rolling belt (driven by a servo-controlled motor) with an adaptable speed and angle profiles, enabling forced exercise training and accurate testing of fatigue in laboratory animals (13, 14). Treadmill System is a fully computerized automatically controlled device designed to assess rodent exercise capacity when forced to run. It is provided with a grid that administers an electric shock (power 700 V Dc at a maximum current of 0.4 mA) that forces the animal to run when it stops and two counters to record the parameters necessary to investigate the physical performance. One counter records the distances and the other the number of electrical shocks received by the animals during the experiment.

In our experiment, rats were trained at a speed of 40 cm/second on a 5 degree tilting of the belt, having run as forced exercise on motorized treadmill 10 minutes sessions.

The locomotors performance of rats was quantitatively evaluated by recording the following parameters: distance run, number of electrical shocks and time of delivering the stimulatory shocks (16).

In this behavioral model of forced exercise, the decreasing of time interval of delivering the electric shocks or the reduction of the number of shocks necessary to motivate the animal corresponds to the endurance-enhancing effect induced by the investigated substance (16, 17). Opposite to this, the increase of time interval for stimulating shock delivery or the number of electric shocks may be correlated with the diminution of physical endurance (17).

On the other hand, the increasing of distance run on the rolling belt may be related to an enhancing of endurance to effort and also to a stimulation of motor activity after substances administration (18, 19). In contrast, the reduction of distance run at the same time interval of the experiment signifies the alteration of animal physical performance in the forced locomotion test (18).

The data were presented as +/- standard deviation and significance was tested using the *SPSS Statistics 17.0* for windows software. Analysis of variance (ANOVA one-way method) and *post hoc* Newman-Keuls and Tukey independent tests were used to determine statistical differences between

the investigated groups. P-values less than 0.05 were considered statistically significant compared to control group.

Experimental protocols were implemented according to the recommendations of the"Gr. T. Popa" University Committee for Research and Ethical Issues. Each animal was used once only and the duration of the experiments was kept as short as possible. For ethical reasons, all the animals were sacrificed at the end of the experiment (20).

RESULTS AND DISCUSSION

The treadmill running test is one of the most frequently used behavioral valuable non-invasive method for assessing the effects of imidazoline receptor antagonists on the locomotor activity and endurance capacity of laboratory animals during physical effort. In addition, the treadmill test was used to clarify the movement motivation of rats (21).

Using the behavioral experimental model of forced locomotion we obtained three types of information regarding the animal physical ability. The following parameters recorded during the 10 minute sessions (distance run, number and delivery time of electric shocks) represent essential tools used to estimate the endurance capacity, and an important modality to corroborate and validate the biological significance of data (14, 22).

During habituation to monitorized treadmill, rats treated with idazoxan (3 mg/kbw) showed a significant increase ($p<0.05$) of distance run (248.17±27.25 meters) compared to the control group (205.75±39.60 meters) (fig. 1).

Intraperitoneal administration of efaroxan 1 mg/kbw lead to an increase in distance run (220.00±19.09 meters), statistically significant ($p<0.05$) compared to the control group (205.75±39.60 meters) in the same behavioral model (fig. 1).

The mean distance run by rats treated with efaroxan was shorter than of rats treated with idazoxan, in the same time interval in the experiment (tab. I).

These results proved the ability of imidazoline receptor antagonists idazoxan and efaroxan to improve fatigue resistance during forced running on this behavioral experimental model.

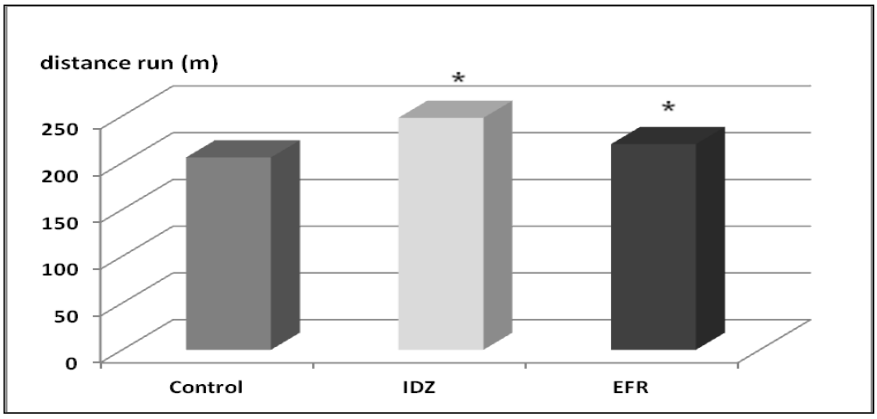

Fig. 1. The effects of idazoxan and efaroxan on distance run in the treadmill exercise test. Each value is the mean ± SEM of distance run (meters) for seven rats. *$p<0.05$, **$p<0.01$ *vs.* control group.

TABLE I
Descriptive statistics – distance run

	N	Mean	Std. Deviation	Std. Error	Min	Max	Sig
Control	8	205.75	39.60	14.00	116.00	233.00	
IDZ	8	248.17	27.25	9.23	163.00	252.00	**0.031***
EFR	8	220.00	19.09	6.75	179.00	239.00	**0.046***

* Mean difference significant at .05 level; ** Mean difference significant at .01 level.

TABLE II
Descriptive statistics – number of electric shocks

	N	Mean	Std. Deviation	Std. Error	Min	Max	Sig
Control	8	96.88	79.90	28.25	26.00	273.00	
IDZ	8	78.36	61.65	31.35	11.00	216.00	**0.039***
EFR	8	85.38	84.59	29.91	6.00	279.00	**0.047***

* Mean difference significant at .05 level; ** Mean difference significant at .01 level.

The administration of idazoxan (3 mg/kbw) was associated with a significant reduction ($p<0.05$) in the number of electric shocks (78.36±61.65) and also in delivery time of shocks (25.28±19.23 seconds) compared to control group (96.88±79.90, 32.64±25.57 seconds respectively) (fig. 2.).

The treatment with efaroxan 1 mg/kbw resulted in a significant ($p<0.05$) decreasing of both electric shocks number (85.38±84.59) and delivery time of shocks (28.59±25.35 seconds) compared to control group (96.88±79.90, 32.64±25.57 seconds respectively) (fig. 2, 3).

The effects of idazoxan on these parameters were more intense than those of efaroxan in the forced exercise test (tab. II, III).

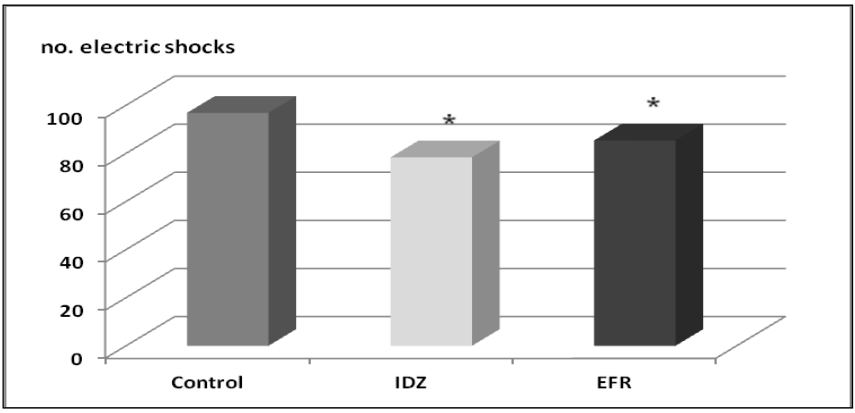

Fig 2. The effects of idazoxan and efaroxan on the number of electrical shocks in treadmill exercise test. Each value is the mean ± SEM of number electric shocks for seven rats. *$p<0.05$, **$p<0.01$ *vs.* control group.

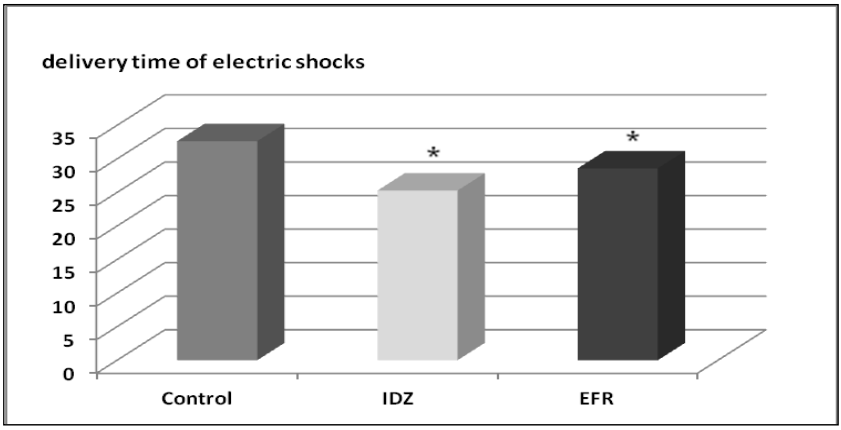

Fig 3. The effects of idazoxan and efaroxan on the delivery time of electric shocks in treadmill exercise test. Each value is the mean ± SEM of distance run (meters) for seven rats. *p<0.05, **p<0.01 *vs.* control group.

TABLE III
Descriptive statistics – delivery time of electric shocks

	N	Mean	Std. Deviation	Std. Error	Min	Max	Sig
Control	8	32.64	25.57	9.04	8.40	74.80	
IDZ	8	27.93	24.83	6.32	7.90	74.40	0,29
EFR	8	28.59	25.35	8.96	9.00	76.00	0,45

* Mean difference significant at .05 level; ** Mean difference significant at .01 level.

CONCLUSIONS

Our experimental investigation demonstrated clear beneficial effects of both imidazoline receptor antagonists idazoxan and efaroxan on endurance exercise performance.

The intraperitoneal administration of idazoxan and efaroxan resulted in an improvement of physical performance and also of endurance capacity in the forced locomotion experimental model in rats.

These findings prove the ability of imidazoline receptor antagonists idazoxan and efaroxan to improve locomotor abilities and to delay fatigue in rats during forced running on this behavioral experimental model. The effects of idazoxan on the recovery of locomotor activity of rats were more intense than those of efaroxan in the treadmill test.

Much more, using this behavioral experimental model, we demonstrated the valuable effect of idazoxan and efaroxan on the improvement of depression-like behavior in rats.

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NEWS

IL-10 AND ET-1 AS BIOMARKERS OF RHEUMATIC VALVE DISEASE

Rheumatic fever (RF) represents a serious public health problem. It is a rheumatic and inflammatory disease of autoimmune origin, which occurs in response to an infection by group A streptococcus. On a global scale, this agent is responsible for approximately 15.6 million annual cases of rheumatic heart disease, with 282,000 new cases and 233,000 deaths each year. From this perspective, health systems face higher expenses with clinical exams, surgeries and frequent hospitalizations due to congestive heart failure. The pathogenesis of RF involves a complex network of genetic, environmental and immunological interactions. Genetic factors predispose individuals to developing autoimmune reactions. Cytokines are proteic molecules, glycosylated or not, that send a range of stimulatory, modulatory or inhibitory signals to the various cells of the immune system. Studies indicate that the inflammatory response in acute RF on cardiac tissues is generated by antigenic mimicry of the protein M leading to an abundant infiltration of CD4+ T cells. This leads to production of inflammatory cytokines (e.g., TNF-α, IL-2, and IL-10), which have a decisive influence on the immune response of patients with rheumatic fever. It is also known that increased levels of Th1 inflammatory cytokines (TNF-α and IFN-γ) and lower levels of Th2 and regulatory cytokine IL-4 lead to maintenance and progression of rheumatic valvulopathy. Endothelin is a highly potent vasoconstrictor peptide. This peptide is composed of 21 amino acids and has three isoforms. The three isoforms are called endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). Endothelin-1 is the subtype predominantly produced by cardiac endothelium. Some studies show gene expression of endothelin in heart valves of patients who underwent surgical valve replacement. Cardiac involvement in acute RF characterizes the most serious and most important of all manifestations of the disease because of the possibility of progressing to chronic rheumatic valvular disease or death. The most common rheumatic mitral valvulopathy is a dual unbalanced dysfunction, i.e, insufficiency and stenosis in different stages of development, which may lead to an indication of surgical repair or replacement of the damaged valve in children and young people in productive age. In this study were compared the levels of some interleukins (TNF-alpha, IL-4 and IL-10) among different patients with RF. In addition, was assessed gene expression of endothelin-1 in native replaced mitral valves (Leão SC, MD; Menezes Lima MR, Menezes do Nascimento H, et al. IL-10 and ET-1 as biomarkers of rheumatic valve disease. Rev Bras Cir Cardiovasc 2014; 29 (1): 25-30).

Doina Butcovan