ORIGINAL ARTICLE

EFFECTS OF TWO IMIDAZOLINE RECEPTOR ANTAGONISTS IN SPONTANEOUS BEHAVIOUR IN RATS

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Abstract

We aimed to investigate the effects of imidazoline receptor antagonists' idazoxan and efaroxan on spontaneous behaviour in rats. Rats were treated intraperitoneally with single doses of either idazoxan (1 mg/kgbw) and efaroxan (3 mg/kgbw) or distilled water (0.3 mL/ 100 g body weight). Locomotor activity and exploratory behaviour of the animals were evaluated using the LE-8811 Actimeter PanLAB device in order to count the number of horizontal, vertical and stereotypic movements. The data were presented as mean ± standard deviation, statistical significance being calculated using the ANOVA test of the SPSS 17.00 Statistics software. Intraperitoneal administration of idazoxan and efaroxan resulted in a reduction of rat horizontal, vertical and stereotypic movements, statistically significant compared to control group. The effects of idazoxan were more intense than those of efaroxan in this experimental behavioural model. We can conclude that the treatment with both imidazoline receptor antagonists was associated with important sedative effects in the Actimeter test in rats.

Rezumat

Studiul a urmărit investigarea efectelor antagoniştilor receptorilor imidazolinici idazoxan şi efaroxan asupra comportamentului spontan la şobolan. Animalele au fost tratate intraperitoneal, în doză unică, fie cu idazoxan (1 mg/kg corp), efaroxan (3 mg/kg corp), sau apă distilată (0,3 mL/ 100 g corp). Activitatea locomotorie şi comportamentul explorator al animalelor au fost evaluate utilizând dispozitivul LE-8811 Actimeter PanLAB pentru a contoriza numărul mişcărilor orizontale, verticale sau stereotipe. Datele au fost exprimate ca medie ± deviaţia standard, iar semnificaţia a fost interpretată utilizând testul ANOVA din programul SPSS 17. Administrarea intraperitoneală de idazoxan şi efaroxan a avut ca rezultat reducerea mişcărilor orizontale, verticale şi stereotipe semnificativ statistic comparativ cu lotul martor. Efectele idazoxanului au fost mai intense decât cele ale efaroxanului în acest model experimental de comportament. Se poate concluziona faptul că tratamentul cu ambii antagonişti ai receptorilor imidazolinici s-a asociat cu efecte sedative importante la testul Actimetrului la şobolan.

Keywords: idazoxan, efaroxan, behaviour, locomotor activity

Introduction

Since their first identification in 1984, the imidazoline receptors have been intensively studied by research groups around the world. Depending on their location, these receptors are both central and peripheral, influencing many physiological and pathological processes by mechanisms that are still to be clarified [1]. The activation of imidazoline receptors leads to several phenomena in the human body: inhibitory sympathetic action; neuroprotective effects and mediation of the nociceptive sensitivity, insulinotropic activity, mediation of the addictive phenomenon and antidepressant activity [1, 3, 6]. Numerous research studies have revealed the implication of imidazoline receptors in several other patho-physiological processes, such as: cell adhesion and proliferation, regulation of adipose tissue formation and feeding behaviour, inflammatory processes, adaptation and response to stress and psychiatric diseases, pathological pathways involved in epilepsy and processes of neoplastic cell transformation [1, 8, 17, 18]. The literature reported data agree on the existence of three types of imidazoline receptors: I_1 (mediating the sympatho-inhibitory actions to decrease blood

pressure), I_2 (modulating the level of central monoamines, with potential antidepressant and antinociceptive activity) and I_3 (regulating the insulin secretion of pancreatic beta Langerhans cells) [1, 2, 7]. The best known endogenous agonist of imidazoline receptors is agmatine, which is non-selective, binding to all three types of receptors and also to other receptors, such as the NDMA receptor and alpha-2 adrenergic receptors [8, 9, 18]. Numerous other agonists have been reported, very few of them being selective on the imidazoline receptors; also, some potent antagonists are known, many being still in clinical trials and not yet implemented in therapy. Recently, the neuro-pharmacology studies regarding the implication of I_2 receptors on modulating behaviour, cognitive functions and locomotor activity have focused on the research of two I_2-ligands: efaroxan and idazoxan, both drugs being antagonists of the I_1, respectively I_2 receptors and also antagonists of the alpha-2 adrenergic receptors [5, 14].

Our purpose was to investigate the effects of imidazoline receptor antagonists' idazoxan and efaroxan on spontaneous behaviour in rats.

Modification in spontaneous locomotor activity of laboratory animals represents a resourceful analysis method to evaluate central neuro-behavioural effects of the investigated substances. The exploratory behaviour assessment may be a suitable screening method to evaluate the effects of various pharmacologic active substances on animal spontaneous locomotor activity [13].

Materials and Methods

White Wistar rats (200–250 g) were randomly assigned to 3 groups of 7 animals each. The rats were housed two per cage in standard laboratory conditions with controlled temperature and humidity (22°C, respectively 60 ± 5%) in a 12 h light/dark cycle, receiving food and water *ad libitum*.

The rats were treated intraperitoneal with single doses of either idazoxan (1 mg/kgbw), efaroxan (3 mg/kgbw, Sigma-Aldrich Co., Germany) or distilled water (0.3 mL/100 g body weight). The drugs were diluted in distilled water and prepared *extempore*.

Locomotor activity and exploratory behaviour of laboratory animals were monitored using the *LE-8811 Actimeter PanLAB* home cage activity device. This device is equipped with three transparent cages, each with eight infrared lights located in a frame around the cage and connected to silent electronic counters. The apparatus is composed by a two-dimensional (horizontal and vertical axes) square frame, a frame support and a control unit. The lower tier records horizontal movements, while the upper tier records vertical movements. The frames are connected directly through a *SeDaCom* computer software, which allows easy exportation of data in a format compatible with the Excel program for Windows. Values are recorded electronically and displayed according to prior programming an electronic system for data processing.

The animals were kept in the test room for habituation for 1 hour before the test. After injection of the vehicle or tested substances, the animals were placed in the device cages and each movement produced a signal caused by variation of inductance and capacity of the apparatus resonance circuit. These signals were automatically converted to numbers. The activity of each rat was automatically recorded for twenty minutes.

The experimental protocol was conducted following the recommendations of the 'Gr. T. Popa' University Committee for Research and Ethical Issues Guidelines according to the ethical standards of the European Community. In particular, the duration of the experiments was kept as short as possible. For ethical reasons, each animal was used once only and was sacrificed immediately after the experiment [15].

Statistical analysis

The data for each measurement were expressed as mean ± standard deviation and graphically represented. Data analyses were performed using SPSS 17.0 software for Windows. Following the assurance of a normal distribution of data, ANOVA one-way analysis of variance with the Newman-Keuls and Tukey post-hoc tests were used for multiple comparisons. P-values less than 0.05 were considered statistically significant compared to control group.

Results and Discussion

The *Actimeter test* is a behavioural experimental model that offers information regarding the animal spontaneous motor activity (total, horizontal, vertical and respectively stereotypic movements) [13, 17]. The number of total movements represents an element that reflects the general animal behaviour during a certain period of time, being a reliable tool for confirmation and validation of biologically important data [2, 16].

Intraperitoneal injection with idazoxan (1 mg/kgbw) or efaroxan (3 mg/kgbw) was associated with a significant decrease of spontaneous total activity (1296.14 ± 530.96, respectively 2469.71 ± 1601.10), compared to the group treated with distilled water (3125.0 ± 1047.17). The effects of idazoxan were more intense than those of efaroxan in the same time interval of determination in the Actimeter test (Figure 1).

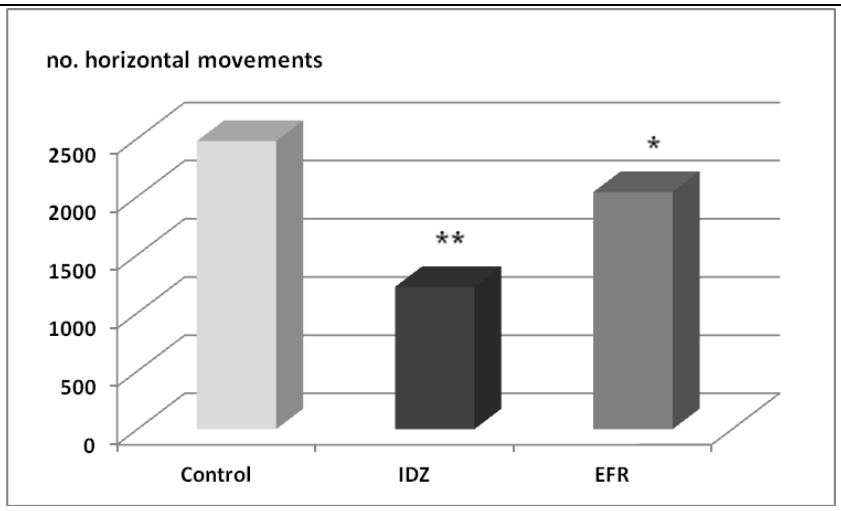

Figure 1.
The effects of idazoxan and efaroxan on the number of horizontal movements in the Actimeter test. Each point represents the mean ± SEM of latency time (seconds) for seven rats. *p < 0.05, **p < 0.01 *vs*. control

The treatment with idazoxan resulted in a reduction of the number of horizontal plane movements (1224.14 ± 436.65), statistically significant (p < 0.01) compared to control animals (2477.43 ± 946.79). The animals treated with efaroxan showed a significant (p < 0.05) diminution of horizontal movements (2039.0 ± 1327.23) compared to the group that received distilled water (2477.43 ± 946.79), in the Actimeter test (Figure 1). The effects of idazoxan on the number of horizontal movements were more accentuated than those of efaroxan in the same time interval in the experiment (Figure 1).

The administration of idazoxan induced a significant (p < 0.01) diminution of vertical locomotor activity (72.0 ± 99.12), compared to the control group (647.57 ± 373.3). Efaroxan decreased the number of vertical plane movements (430.71 ± 313.01), statistically significant (p < 0.05) compared to the control group (647.57 ± 373.3) (Figure 2). Its effects were less intense than those of idazoxan in the same behavioural experimental model in rats (Figure 2).

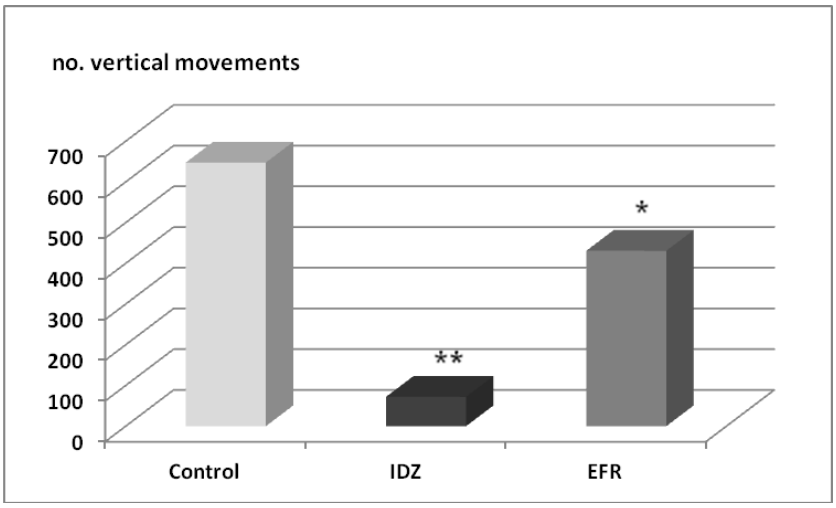

Figure 2.
The effects of idazoxan and efaroxan on the number of vertical movements in the Actimeter test. Each point represents the mean ± SEM of latency time (seconds) for seven rats. *p < 0.05, **p < 0.01 *vs*. control

Intraperitoneal injection of idazoxan resulted in a reduction of rats stereotypic movements (944.14 ± 101.46), statistically significant (p < 0.05) compared to the control group (1088.71 ± 157.26); the effects were more intense than those of efaroxan, which also induced a decrease of stereotypic movements (1013.0 ± 152.28), statistically significant (p < 0.05), compared to the control group (1088.71 ± 157.26) (Figure 3).

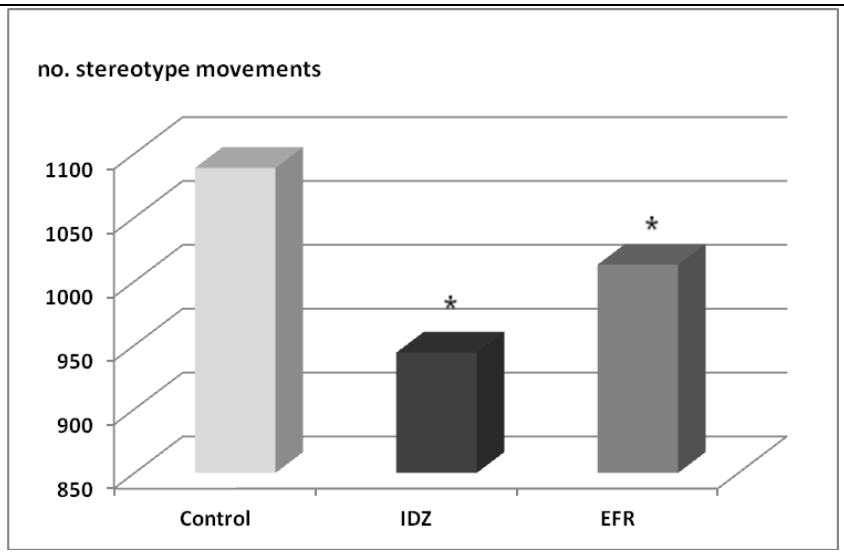

Figure 3.
The effects of idazoxan and efaroxan on the number of stereotypic movements in the Actimeter test. Each point represents the mean ± SEM of latency time (seconds) for seven rats. *p < 0.05, **p < 0.01 *vs*. control

Experimental studies conducted on laboratory animals have revealed that efaroxan and idazoxan completely block the anti-compulsive-like effect of agmatine, indicating the involvement of imidazoline receptors in anxiety and obsessive compulsive disorders [4]. Other experimental studies suggest agmatine and imidazoline receptors as a potential therapeutic target for the treatment of depressive disorders, demonstrating that efaroxan and idazoxan blocked the antidepressant-like effect of bupropion and its synergistic activity when associated with agmatine [12]. An experimental study conducted in 2014 has revealed the implications of imidazoline receptors on inhibitory performance learning activity of rats [4]. Retrodialysis of the I_2 antagonist idazoxan proved to potentiate the release of norepinephrine induced by footshock application in the basolateral amygdala of rats [5].

The monitoring of horizontal movements is useful to evaluate the general exploration behaviour of the animal in contact with a new environment [11]. The rearing behaviour or the vertical plane movement represents the animal trying to ascend on the walls of home cage device, behaviour that could be assimilated with escape attempts, indirectly correlated with fear and anxiety activities. The stereotypic movements suggest the self-maintenance of animal personal hygiene (paw-licking actions, hair combing and nose cleaning, auto-grooming behaviour) [2].

We have demonstrated that both idazoxan and efaroxan determined a diminution of exploratory activity and a decrease of the escape attempts, thus corresponding to a reduction of rat spontaneous behaviour and of locomotor activity, assimilated somehow to sedative effects [13]. At the same time a declining of exploratory and self-maintenance animal behaviour were observed, actions that can be correlated to a decrease of anxious behaviour [10].

These results are simply indicative because the data cannot be clinically extrapolated directly, taking into account the huge differences of behaviour, cognitive performances and locomotor activity coordination between rats and humans.

Conclusions

We can conclude that the treatment with idazoxan suppressed exploratory activity, rearing and self-maintenance behaviour of the experimental animals, its effects being stronger than those of efaroxan in the Actimeter test.

These observations provide evidence supporting the involvement of imidazoline receptors in the mediation of exploratory activity, motor coordination and spontaneous behavioural changes of the laboratory animal.

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