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# Effect of the Reference Imidazoline Drugs, Clonidine and Rilmenidine, on Rat Eye Pupil Size Confirms the Decisive Role of $\alpha_2$-Adrenoceptors on Mydriasis

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**Abstract:** The *in vivo* rat mydriasis model provides more information on ligand-receptor interactions than *in vitro* studies. It allows assessment which receptors ($\alpha_2$-adrenergic or imidazoline) participate in mydriatic effect produced by imidazoline compounds, regarding the agonistic and antagonistic properties and the strength of the reference ligands action. The reference imidazoline agents can be tested within a wide range of doses, *in vivo* in the animals. The experiments were performed to assess the mydriatic effects of clonidine and rilmenidine with and without pretreatment of rats with yohimbine, a reference $\alpha_2$-adrenoceptor antagonist. Additionally, compound AGN 192403, regarded as a selective antagonist of $I_1$-imidazoline receptors was used in experiments. However, the previous data indicated that AGN 192403 was devoid of both agonist and antagonist activity in some functional assays. As a result of further investigation, AGN 192403 has been reported as an antagonist to bind selectively at the $I_1$ imidazoline binding site. The $\alpha_2$-adrenomimetic potency of clonidine appeared to be stronger than rilmenidine. Maximum mydriatic effect for clonidine and rilmenidine was found at doses of 30 and 1000 μg $kg^{-1}$, respectively. Yohimbine administration caused parallel shift of the dose-effect curve for both rilmenidine and clonidine to the right. Preliminary experiments with AGN 192403 seem to confirm the hypothesis that the involvement of $I_1$-imidazoline receptors in mydriatic effects of imidazolines is marginal, if any. The present study shows that rilmenidine has about 30 times weaker affinity to central $\alpha_2$-adrenoceptors than clonidine. Yohimbine inhibits pupil dilation evoked by rilmenidine and clonidine. That confirms the decisive role of $\alpha_2$-adrenoceptors in mydriatic effect and suitability of the Rat Pupil Mydriasis Model for studies of receptor selectivity of the centrally acting drugs.

**Key words:** Mydriasis, $\alpha_2$-adrenoceptor, imidazoline receptor, clonidine, rilmenidine, AGN 192403

## INTRODUCTION

The imidazoline derivatives are showing a variety of pharmacological effects, including hypotension, sedation, bradycardia, hypothermia but also mydriasis. Some of these effects, like hypotension or bradycardia, are correlated with the mydriatic effect in dose-dependent manner (Nasal *et al*., 1995). The multifuncional action of these compounds arises from the fact that they have affinity to different types of receptors, mainly to the $\alpha_2$-adrenoceptors which are currently divided into A, B, C and D subtypes (Calzada and Artinano, 2001) as well as to imidazoline $I_1$ and $I_2$ receptors but also to the cholinergic, histaminergic and dopaminergic receptors. Imidazoline moiety occurs in many therapeutic agents, such as antihistamines or general anaesthetics (Brenner and Stevens, 2010). Actually, importance of imidazoline derivatives is extended for treatment of hypotension with accompanying diseases; arrhythmia, heart failure, hyperlipidemia etc. (Stabile *et al*., 2011).

Literature data indicate that imidazoline $I_1$ receptors may mediate at least some of the central nervous system (CNS) effects of the clonidine-like drugs (Ernsberger and Haxhiu, 1997; Guyenet, 1997). Some antihypertensive imidazoline drugs both decrease sympathetic tone by stimulating CNS $\alpha_2$-adrenoceptors and produce mydriasis in some species, like cats, rats and mice, when administered either topically (Walland and Kobinger, 1971) or intravenously (Koss and San, 1976; Gherezghiher and Koss, 1979).

Edinger-Westphal nucleus plays an important role in the vision mechanism. It begins with the preganglionic parasympathetic fibers that control the ciliary body and pupillary sphincter muscle (Yu and Koss, 2005). Within it

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there is a large number of central postsynaptic $\alpha_2$-adrenergic receptors. Their stimulation causes direct mydriatic effect on the pupil of the eye (Koss, 1986; Szabadi and Bradshaw, 1996).

Mydriatic model in rats proposed by Koss (1986) might be a very useful pharmacodynamic system for assessment of selective CNS $\alpha_2$-adrenergic activity of the agents comprising imidazoline moiety. It may be conveniently applied in preclinical studies to characterize $\alpha_2$-adrenoceptor antagonistic properties of drugs and drug candidates (Yu and Koss, 2005). Additionally, this model may provide means to exclude the participation of the supposed $I_1/I_2$ receptor ligands in the pupil dilation in rats (Yu and Koss, 2005). It may also allow determination of the receptor subtype, through which the analogues of imidazoline drugs exert their effects.

In this study, two reference imidazoline drugs: Clonidine and rilmenidine, were used. They have both been classified as mixed $\alpha_2/I_1$ and $I_1/\alpha_2$-receptors agonists, respectively (Szabo *et al*., 2001). Rilmenidine is a centrally acting antihypertensive drug (Koss, 2003) which is claimed to cause less side-effects in comparison to clonidine but it also produces a dose-related mydriasis (Feldman *et al*., 1998). The aim of the study was to extend the basic knowledge on the phenomena observed in the ligand-receptor interactions of clonidine and rilmenidine by testing their action directly and after pretreatment with the specific $\alpha_2$-antagonist: Yohimbine or a selective imidazoline $I_1$-receptor antagonist: AGN 192403. Studies were conducted to check by which receptors, the mydriatic action is mediated and also to optimize the experimental conditions for the pupil diameter measurement.

## MATERIALS AND METHODS

**Animals:** The studies were performed in 20 male Wistar rats weighing 200-300 g. The rats were anaesthetized with pentobarbital sodium (60 mg $kg^{-1}$, i.p.).

**Drugs:** Clonidine hydrochloride (Tocris, Bristol, United Kingdom), Rilmenidine dihydrogen phosphate (Technologie Servier, Neuilly sur Seine, France), Yohimbine hydrochloride (Sigma-Aldrich Technical Services, St. Louis, United States), AGN 192403 (Tocris, Bristol, United Kingdom).

**Pupil diameter measurement:** Pupil diameter measurement was carried on by adapting the Koss method (Koss, 1986), pupil diameter was measured with an accuracy of 0.10 mm at the moment of maximum width of the pupil. Measurements were performed by using a stereoscopic microscope (MST 132 LAB TK PZO, Warszawa, Poland), equipped with a scale and an external light source.

A green filter was used to both eliminate the evoked by the light reaction of the pupil and to enhance the image contrast of the iris. All the experiments were performed in a darkened room at fixed light conditions.

The initial value of the pupil diameter, before the administration of 0.9% NaCl solution and studied drugs, was about 0.70±0.1 mm. The substances were dissolved in 0.9% NaCl solution and administered through the femoral vein (1 mL $kg^{-1}$) in cumulative doses at 5 min intervals. After 3 min from administration of each dose, the response of the pupil was measured.

Yohimbine was administered through the rat femoral vein at a dose of 1.5 mg $kg^{-1}$ 10 min before the first dose of clonidine or rilmenidine. Similarly, AGN 192403 was administered at a dose of 5 mg $kg^{-1}$.

**Statistical analysis:** Dose-mydriatic effect curves after administration of imidazoline drugs studied to rats were constructed applying GraphPrism, version 5.0 software. The doses of the agents studied which produced 50% maximum mydriatic effect, $ED_{50}$, were also calculated with using this program.

## RESULTS

The results (mean of 5 experiments) are shown in the form of curves illustrating the dependence of mydriatic effect (in millimeters) on the logarithmically increasing dose (μg $kg^{-1}$) of clonidine or rilmenidine (Fig. 1-3). Other experiments were also performed to study the influence of the intravenously administered 0.9% NaCl solution on the mydriatic effect. The 0.9% NaCl solution was administered at the same time intervals and volumes as the tested compounds. No pupil reaction was observed which confirmed assumption that 0.9% NaCl solution, used as a solvent for the tested ligands, did not evoke mydriasis in rats by itself. An experimental group of rats were used to study the dose-dependent mydriasis after intravenously administered AGN192403 alone, given in cumulative doses (0.1-5 mg $kg^{-1}$) at 5 min intervals. For a more quantitative assessment of the AGN 192403 effect on the pupil dilation in rats, a maximal dose of rilmenidine, 3000 μg $kg^{-1}$ was administered and then after 15 min, consecutively increasing cumulative doses of AGN 192403 were given i.v., at 5 min intervals. The results show

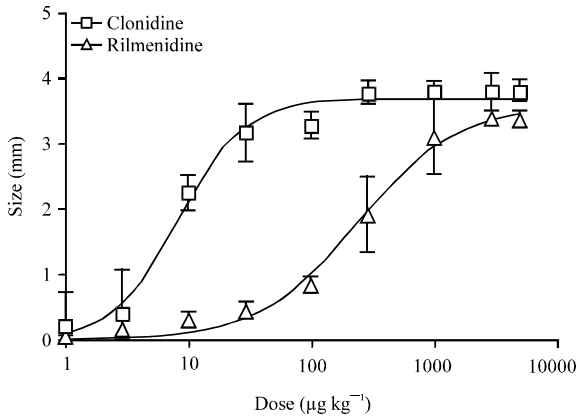


Fig. 1: Effects of clonidine and rilmenidine on the size of the pupil in rats

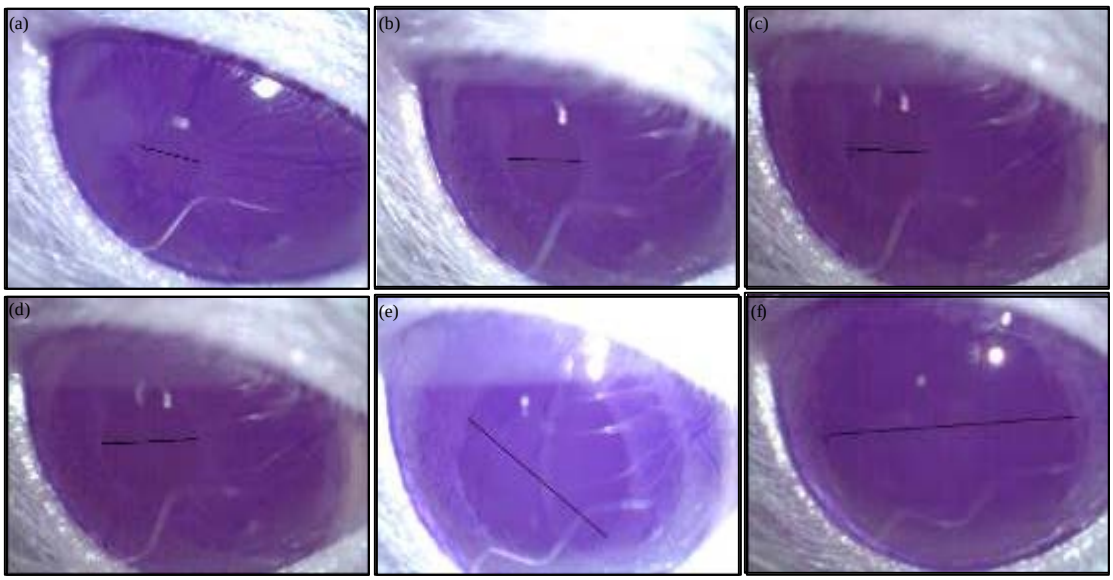


Fig. 2(a-f): Changes in pupil diameter of the rats after administration of clonidine in cumulative doses, (a) Control value, (b) 1, (c) 3, (d) 5, (e) 10 and (f) 30 µg kg$^{-1}$

slight mydriatic effect observed in rats eye after administration of AGN 192403 alone (Fig. 4). Moreover, AGN 192403 did not produce a dose-dependent reversal of rilmenidine-induced mydriasis even at the highest dose (Fig. 5).

**Influence of 0.9% NaCl solution and AGN 192403 alone on pupil diameter:** The 0.9% NaCl solution was administered intravenously in cumulative doses, at 5 min intervals. The NaCl solution had no influence on the width of the rats pupil. The average value of the pupil diameter, expressed in mm, was approximately 0.68±0.14 mm. Thus, intravenous administration of 0.9% NaCl had no effect on the size of the pupil.

AGN 192403 was administered intravenously in increasing cumulative doses (0.1-5 mg kg$^{-1}$) at 5 min intervals. Slight mydriatic effect was observed in rats after injection of cumulative doses of AGN 192403. Therefore, it can be assumed that AGN 192403 may act as a weak or partial agonist of $\alpha_2$-adrenergic receptor.

**Pupillary effects of AGN 192403 administered i.v. after dilation response evoked by rilmenidine:** Rats were administered a maximal mydriatic dose of rilmenidine (3000 µg kg$^{-1}$) intravenously and then after 15 min, increasing cumulative doses (0.1-5 mg kg$^{-1}$) of AGN 192403 were injected. It appeared that

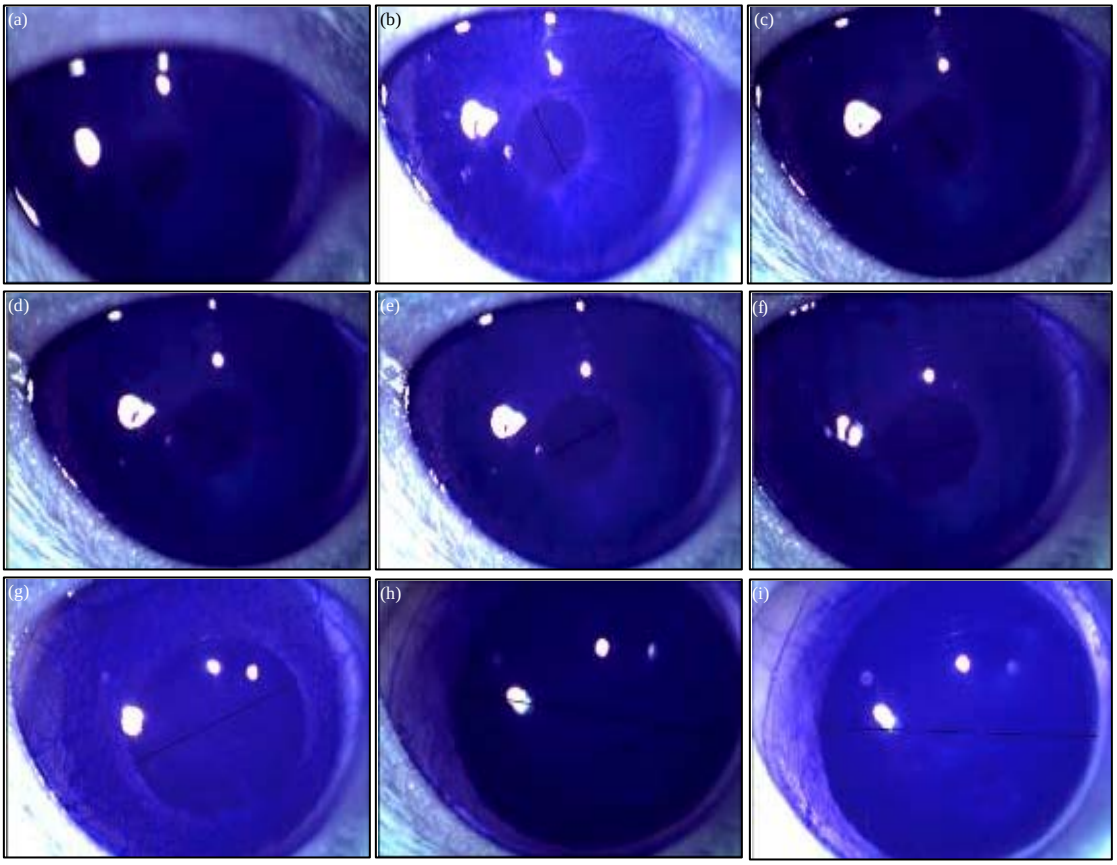


Fig. 3(a-i): Changes in pupil diameter of the rats after administration of rilmenidine in cumulative doses, (a) Control value, (b) 1, (c) 3, (d) 10, (e) 30, (f) 100, (g) 300, (h) 1000 and (i) 3000 μg $kg^{-1}$

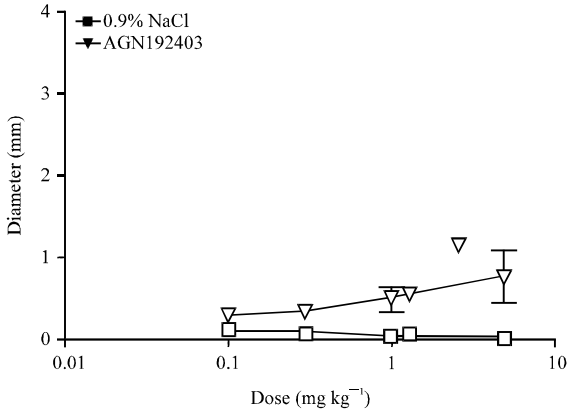


Fig. 4: Influence of 0.9% NaCl solution and AGN192403 on pupil diameter

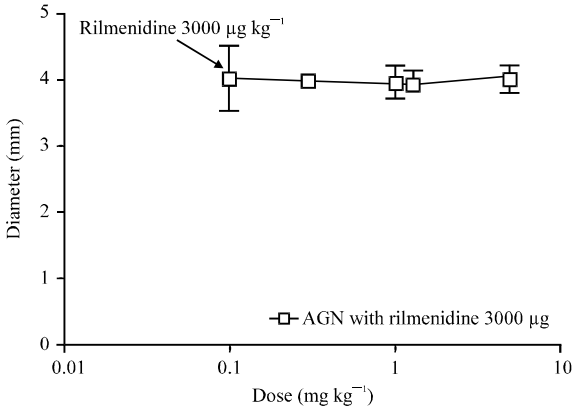


Fig. 5: Pupillary effects of administration of increasing cumulative doses of AGN 192403 (0.1-5 mg $kg^{-1}$) in rats where pupils were previously dilated by rilmenidine (3000 μg $kg^{-1}$ i.v.)

AGN 192403, even at the highest dose, had no influence to reverse the dose-dependent mydriasis, evoked by rilmenidine. This indicates that AGN 192403 did not antagonize pupillary effects in rats evoked by rilmenidine through activation of $\alpha_2$-adrenergic receptors.

**Effects of clonidine and rilmenidine on the size of the pupil in rats**

**Clonidine:** Clonidine was administered intravenously in cumulative doses of 1, 3, 5, 10, 30, 50, 100 and 300 μg $kg^{-1}$. The dose-related mydriatic effect of clonidine is shown in Fig. 4.

The maximum mydriatic effect after administration of cumulative doses of clonidine, as compared with the control value, was 3.61±0.15 mm. Maximum effect occurred at a dose of 29.51 μg $kg^{-1}$. The $ED_{50}$ value was 11.31±0.55 μg $kg^{-1}$.

Mydriasis occured 1 min after administration of clonidine through the femoral vein and was of a long duration (more than 1 h). Figure 3 shows dependence of mydriatic effects on the used doses of clonidine.

**Rilmenidine:** Rilmenidine was administered to the rats intravenously in cumulative doses of 1, 3, 10, 30, 100, 300, 1000 and 3000 μg $kg^{-1}$ and evoked dose-dependent pupil dilation (Fig. 5). Figure 3 presents the dose-mydriatic effects curve for this agent.

The maximum mydriatic effect after administration of cumulative doses of rilmenidine as compared with the control value, was 3.88±0.27 mm. This effect occurred at a dose of 1000 μg $kg^{-1}$. The calculated $ED_{50}$ value was 273.1±1.2 μg $kg^{-1}$.

Likewise clonidine, mydriasis effect was observed within the first minute after rilmenidine administration.

**Mydriatic effect of clonidine and rilmenidine in yohimbine pretreated rats**

**Mydriatic effect evoked by clonidine in the yohimbine-pretreated rats:** Yohimbine, an $\alpha_2$-adrenoceptor antagonist, was administered (1.5 mg $kg^{-1}$) through the rat femoral vein 10 min before administration of the following cumulative doses of clonidine: 1, 3, 5, 10, 30, 50, 100 and 300 μg $kg^{-1}$. The progress of mydriatic effects observed is presented in Fig. 6. The maximum pupil dilation occured at a dose of 79.43 μg $kg^{-1}$ and was 3.76±0.08 mm.

Administration of yohimbine caused a parallel shift to the right of the dose-mydriatic effect curve for clonidine (Fig. 7). The calculated $ED_{50}$ value was 35.45±1.12 μg $kg^{-1}$.

**Mydriatic effect evoked by rilmenidine in the yohimbine-pretreated rats:** Yohimbine was given at a dose of 1.5 mg $kg^{-1}$ into the rat femoral vein 10 min before the administration of cumulative doses of rilmenidine: 1, 3, 10, 30, 100, 300, 1000 and 3000 mg $kg^{-1}$.

The progress of mydriatic effects observed is shown in Fig. 8. The maximum pupil dilation occured at a dose of 2754 mg $kg^{-1}$ and was 3.72±0.4 mm.

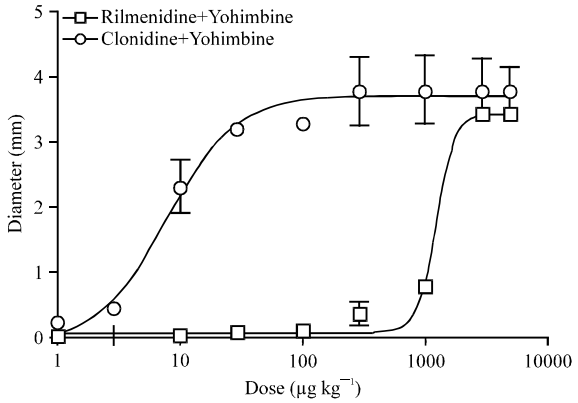


Fig. 6: Mydriatic effect evoked by clonidine and rilmenidine in yohimbine pretreated rats

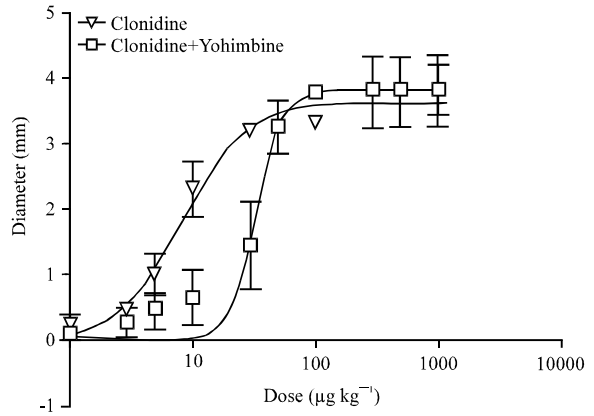


Fig. 7: Comparison of the mydriatic effect evoked by clonidine alone and in yohimbine pretreated rats

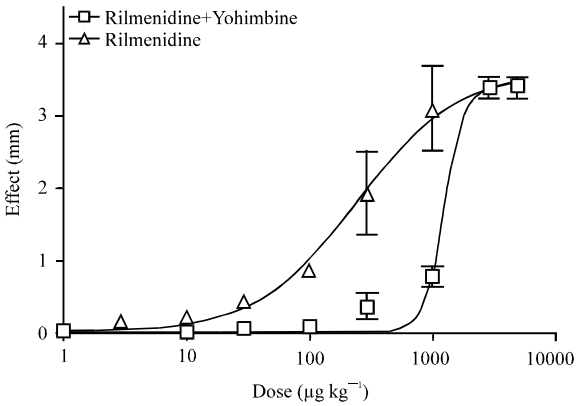


Fig. 8: Comparison of the mydriatic effect evoked by rilmenidine alone and in yohimbine pretreated rats

Yohimbine administration caused parallel shift to the right of the curve, illustrating dependence of the pupil dilation on the dose of rilmenidine (Fig. 8). The $ED_{50}$ value was 1262±1.23 μg $kg^{-1}$.

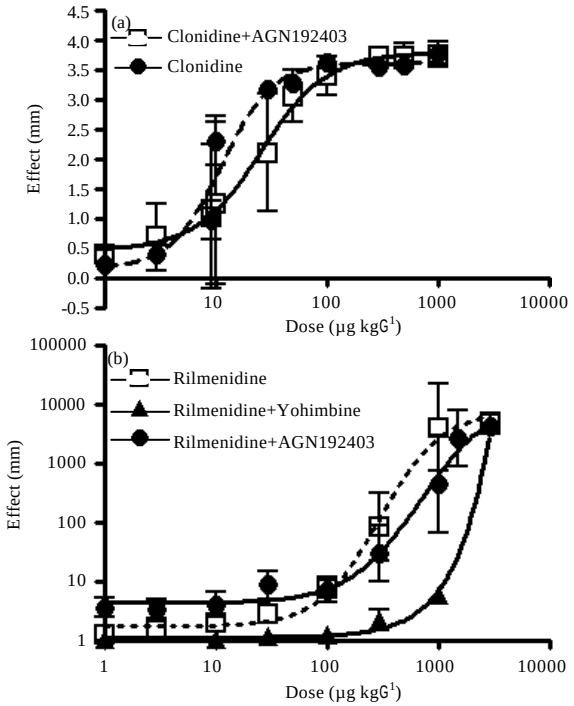


Fig. 9(a-b): Comparison of the mydriatic effect evoked by (a) Clonidine alone and in AGN192403 pretreated rats and (b) Rilmenidine alone and AGN192403 pretreated rats

**Mydriatic effect of clonidine and rilmenidine in the AGN 192403 pretreated rats**

**Mydriatic effect evoked by clonidine in the AGN 192403 pretreated rats:** AGN 192403, a selective imidazoline $I_1$-receptor antagonist, was administered (5 mg $kg^{-1}$) through the rat femoral vein 10 min before administration of the following cumulative doses of clonidine: 1, 3, 5, 10, 30, 50, 100 and 300 µg $kg^{-1}$. The maximum pupil dilation occured at dose 245.47 µg $kg^{-1}$ and was 3.69±0.1 mm.

Administration of AGN 192403 caused a parallel shift to the right of the dose-mydriatic effect curve for clonidine (Fig. 9a). The calculated $ED_{50}$ value was 25.89±3.4 µg $kg^{-1}$.

**Mydriatic effect evoked by rilmenidine in the AGN 192403 pretreated rats:** AGN 192403, a selective imidazoline $I_1$-receptor antagonist, was administered (5 mg $kg^{-1}$) through the rat femoral vein 10 min before administration of the following cumulative doses of rilmenidine: 1, 3, 10, 30, 100, 300, 1000 and 3000 µg $kg^{-1}$. The maximum pupil dilation occured at dose 3236 µg $kg^{-1}$ and was 3.73±0.23 mm.

Administration of AGN 192403 caused a slight parallel shift of the curve to the right of the dose-mydriatic effect for rilmenidine (Fig. 9b). The calculated $ED_{50}$ value was 698.5±5.76 µg $kg^{-1}$.

Table 1: Pharmacometric data from the experiments on rat pupil mydriasis

| | Dose at which the maximal mydriatic effect occured (µg $kg^{-1}$) | Emax (mm) | $ED_{50}$ (µg $kg^{-1}$) |
|---|---|---|---|
| Clonidine | 29.51 | 3.61±0.15 | 11.31±0.55 |
| Clonidine with Yohimbine | 79.43 | 3.76±0.08 | 35.45±1.12 |
| Clonidine with AGN192403 | 245.47 | 3.69±0.10 | 25.89±3.4 |
| Rilmenidine | 1000 | 3.88±0.27 | 273.1±1.2 |
| Rilmenidine with Yohimbine | 2754 | 3.72±0.40 | 1262±1.23 |
| Rilmenidine with AGN192403 | 3236 | 3.73±0.23 | 698.5±5.76 |

**Comparison of the mydriatic effects evoked by imidazoline derivatives:** Comparison of the mydriatic effects evoked by administration of the cumulative doses of imidazoline derivatives (clonidine and rilmenidine) and influence of yohimbine or AGN 192403 on mydriatic effect is presented on the graphs (Fig. 9a, b). Numerical values of pharmacodynamic parameters of clonidine and rilmenidine regarding activity on rat iris are presented in Table 1.

## DISCUSSION

Apart from a few compounds used in medicine (clonidine, moxonidine, rilmenidine), the objects of research involving the rats mydriasis model are mostly other imidazoline agents, classified as $\alpha_2$-adrenoceptors agonists. Literature data on the mydriatic effects caused by the new ligands of this receptor are mostly based on the results of radioisotopic *in vitro* assays which have demonstrated an affinity of the agents to both the $\alpha_2$-adrenergic receptors and to the imidazoline-$I_1$ receptors (Dardonville and Rozas, 2004). However, using *in vitro* radioisotope labelling technique to study new imidazoline derivatives does not suffice to decide, whether the agent studied has an agonistic or antagonistic property. Thus, for an objective classification of imidazoline ligands, the pharmacological *in vivo* test is necessary.

Mydriatic model in rats, applied to imidazoline compounds, may provide especially useful information on ligand-receptor interactions. That simple method, consisting in measuring pupil diameter of the rat eye, has many advantages in comparison to the other pharmacological methods examining the interactions of ligands with central $\alpha_2$-adrenoceptors. It provides an opportunity to perform the experiments *in vivo* in the whole animal, with testing an individual imidazoline agent in a wide range of doses (from a few µg $kg^{-1}$ to several mg $kg^{-1}$). The method allows to test both the agonistic and antagonistic properties of the ligands studied.

Also, it makes possible to obtain reliable dose-effect curves for a given compound and to statistically evaluate the results obtained from experiments carried out on 5-7 rats which normally survive the test.

Previous studies in the laboratory on a series of imidazoline drugs demonstrated that $\alpha_2$-adrenoceptor agonists evoked both mydriasis and blood platelet aggregation. The correlation between mydriatic activity and both the hypotensive and the bradycardic activity was also found. Mydriatic activity but not the platelet aggregation *in vitro*, depended on lipophilicity of the drugs studied. Hence, it could be hypothetized that the $\alpha_2$-adrenoceptors on the surface of blood platelet are easily accessible *in vitro* for the drugs studied. On the other hand, an appropriate lipophilicity is necessary for the imidazoline derivative to access the mydriasis-controlling receptors in CNS *in vivo*. It has also been found that the human platelet antiaggregatory activity of imidazolines does not correlate with neither mydriatic nor cardiovascular activity (Nasal *et al*., 1995).

Physiological effects evoked by imidazoline derivatives acting on the imidazoline and/or $\alpha_2$-adrenergic receptors are often difficult to distinguish. There are many methodological difficulties that occur during the experiments, especially when intracerebral administration of the compounds to the laboratory animals is necessary. Because imidazoline ligands show affinity to both the $\alpha_2$-adrenoceptors and imidazoline ($I_1$/$I_2$) receptors, it is hard to demonstrate which type of receptor is responsible for the particular physiological reaction observed.

The representative imidazoline derivative drugs studied in the present study, clonidine and rilmenidine, have evidently different affinity as regards induction of rat eye pupil mydriasis and hence toward the $\alpha_2$-adrenoceptors. The consistent mydriatic effect of these drugs in rats was observed if examined individually and if tested after pretreatment of the animals with yohimbine (1.5 mg kg$^{-1}$ i.v.) or AGN 192403 (5 mg kg$^{-1}$ i.v.).

Clonidine, well known $\alpha_2$-adrenoceptor agonist, has been reported to elicit 200 times stronger selectivity to the $\alpha_2$-adrenoceptor than to the $\alpha_1$-adrenoceptor subtype (Dziubdziela and Jalowiecki, 2002). However, clonidine has also some affinity for imidazoline receptors and among its congeners there are agents that cause mydriasis in certain species of animals, like rats, cats and mice, by acting on CNS (Yu and Koss, 2005).

Rilmenidine is classified as a centrally acting antihypertensive drug of the second generation. It has a marked affinity to $I_1$-imidazoline receptors but has also been reported to stimulate $\alpha_2$-adrenoceptors (Koss, 2003).

Clonidine causes a dose-dependent pupil dilation in rats after i.v., administration to the animals at the dose as low as 1 μg kg$^{-1}$. This compound has been confirmed here to be a good reference agent for other imidazoline derivatives having affinity to $\alpha_2$-adrenoreceptors (Koss, 1986; Berridge *et al*., 1983).

The value of maximum clonidine mydriatic effect, obtained in this study, was consistent with the literature reports. Also, the value of the dose producing 50% of the maximum pupil dilation, $ED_{50}$, for clonidine ($ED_{50}$ 11.31±0.55 mg kg$^{-1}$) from these studies, was similar to the values from literature (Yu and Koss, 2005; Koss, 2003). Experiments with clonidine and rilmenidine performed in the rats pretreated and non-pretreated with yohimbine, showed that yohimbine causes paralel shift of dose-effect curves to the right. That clearly supports the participation of $\alpha_2$-adrenergic receptors in mydriatic action of clonidine and rilmenidine (Fig. 7, 8).

Rilmenidine administered intravenously caused mydriatic effect in rats at doses starting from 1 μg kg$^{-1}$ but the effect evoked by the equivalent dose of clonidine was significantly stronger (Fig. 1). The measurements of pupil diameter in rats, after administration of cumulative doses of rilmenidine, confirmed a lower affinity of this compound to the $\alpha_2$-adrenergic receptors located in the central nervous system. The effective dose for rilmenidine ($ED_{50}$ =273.1±1.2 μg kg$^{-1}$) is closely similar to the literature values reported by other authors (Yu and Koss, 2005; Koss, 2003).

Yohimbine, an $\alpha_2$-adrenoceptor antagonist, given to the rats before administration of cumulative doses of clonidine, produced a significant reduction of dilation of pupil's diameter. Maximum mydriatic effect occurred at a dose of 79.43 μg kg$^{-1}$ ($ED_{50}$ =35.45±1.12) (Fig. 1).

Administration of yohimbine to the rats, 10 min before administration of cumulative doses of rilmenidine, resulted in a minor reduction of dilation of pupil diameter. The maximum mydriatic effect occurred at a dose of 2754 μg kg$^{-1}$ ($ED_{50}$ =1262±1.23) (Fig. 6).

Comparison of the results of this study indicates, that rilmenidine has about 30 times weaker affinity to central $\alpha_2$-adrenoceptors than clonidine.

Administration of AGN 192403, an $I_1$-imidazoline receptor antagonist, before administration of agonists allowed to assess the possible involvement of $I_1$-imidazoline receptors in the mydriatic effects of the ligands studied.

AGN 192403, given to the rats at a dose of 5 mg kg$^{-1}$ prior to administration of cumulative doses of clonidine or rilmenidine, did not cause a significant shift of the dose-effect curves of the ligands studied. Maximum mydriasis effect has not changed and it occurred after administration of 10-fold higher dose of clonidine:

$E_{max}$ = 3.69±0.1 mm ($ED_{50}$ =25.89±3.4). Administration of cumulative doses of rilmenidine at the presence of the imidazoline $I_1$-receptor antagonist, did not result in a significant shift of the dose-effect curve for rilmenidine. Maximum mydriasis $E_{max}$ was 3.73±0.23 mm and occurred after administration of a 3-fold higher dose of rilmenidine ($ED_{50}$ =698.5±5.76). Pupillary effect of AGN 192403 given to the rats alone (Fig. 4) indicates that AGN 192403 may have residual agonistic properties toward $\alpha_2$-adrenergic receptors.

Independent pharmacological studies were reported on the selective antagonists of $\alpha_2$-adrenergic receptors: compounds RX 821002 and RS 79948. The results of experiments that were presented with the derivatives of imidazoline, clonidine, rilmenidine and with an $\alpha_2$-adrenoceptor antagonist, yohimbine, confirm the reports of other authors on the impact of imidazoline derivatives on the rat eye pupil dilation (Yu and Koss, 2005; Berridge *et al*., 1983; Christensen *et al*., 1990).

In all the experiments as a solvent for the tested ligands, 0.9% NaCl solution was used. Administration of the same volumes of solvent as were used during the ligands testing, through the rat femoral vein, did not evoke significant changes in pupil size.

Continuation of the present research could be of practical importance for the search for selective $\alpha_2$-agonists possessing no imidazoline receptor activity. Enhanced $\alpha_2$-adrenergic receptor activity should reduce some untoward side effects of a new drug, e.g., elevated intraocular pressure, a condition often associated with glaucoma. The imidazoline derivatives which have mutually similar physicochemical and pharmacokinetic properties, are also known to interact with two classes of receptors, i.e., $\alpha_2$-adrenergic and $I_1$-imidazoline ones. However, differences in the *in vivo* performance of these ligands may result from the stimulation of different types of receptor within the brain (especially within the RVLM region).

The clonidine analogues, well known as antihypertensive drugs, have side effects, such as sedation, mouth dryness, bradycardia, "rebound hypertension", intestinal disorders or libido changes (Tolentino-Silva *et al*., 2000; Van Zwieten *et al*., 1984). The search for drugs more selectively interacting with imidazoline receptors of $I_1$ type (rilmenidine, moxonidine) can result in reduction of the adverse effects of "imidazoline-like" agents and hence in improvement of their pharmacodynamic parameters. It can be noted here that additional benefit possibly arising from the use of imidazoline agents is that they are reported to improve glucose tolerance and to lower the insulin, triglycerides and cholesterol levels (Ernsberger, 2000).

The results of this study show that $\alpha_2$-adrenomimetic potency of clonidine is markedly stronger than $\alpha_2$-adrenomimetic potency of rilmenidine. Maximum mydriatic effect (3.61±0.15 mm) for clonidine occurred at a dose of 29.51 μg $kg^{-1}$, whereas for rilmenidine (3.88±0.27 mm) it appeared at a dose of 1000 μg $kg^{-1}$ (Fig. 3).

The proposed simple method consisting in measuring the width of the pupil of rats eye, has specific advantages. Compared with other *in vivo* and *in vitro* pharmacological methods of testing the interactions of ligands having imidazoline structure with central $\alpha_2$-adrenergic receptors, this model seems to be especially useful for the preclinical *in vivo* studies aimed at identification of the potential selective drugs interacting through $\alpha_2$-adrenoceptors.

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## REFERENCES

Berridge, T.L., B. Gadie, A.G. Roach and I.F. Tulloch, 1983. Álpha$_2$-adrenoceptor agonists induce mydriasis in the rat by an action within the central nervous system. Br. J. Pharmacol., 78: 507-515.

Brenner, G.M. and C.W. Stevens, 2010. Pharmacology. 3rd Edn., Saunders Elsevier, Philadelphia.

Calzada, B.C. and A.A. de Artinano, 2001. Alpha-adrenoceptor subtypes. Pharmacol. Res., 44: 195-208.

Christensen, H.D., M. Mutzig and M.C. Koss, 1990. CNS alpha$_2$-adrenoceptor induced mydriasis in conscious rats. J. Ocular Pharmacol. Ther., 6: 123-129.

Dardonville, C. and I. Rozas, 2004. Imidazoline binding sites and their ligands: An overview of the different chemical structures. Med. Res. Rev., 24: 639-661.

Dziubdziela, W. and P. Jalowiecki, 2002. (Alpha-2-adrenergic receptors and their agonists in anesthetic practice]. Anestezjologia Intensywna Terapia, 2: 135-140, (In Polish).

Ernsberger, P. and M.A. Haxhiu, 1997. The I1-imidazoline-binding site is a functional receptor mediating vasodepression via the ventral medulla. Am. J. Physiol.-Regul. Integr. Comp. Physiol., 273: R1572-R1579.

Ernsberger, P., 2000. Pharmacology of moxonidine: An I1-imidazoline receptor agonist. J. Cardiovasc. Pharmacol., 35: S27-S41.

Feldman, J., H. Greney, L. Monassier, C. Vonthron, V. Bruban, M. Dontenwill and P. Bousquet, 1998. Does a second generation of centrally acting antihypertensive drugs really exist? J. Autonomic Nervous Syst., 72: 94-97.

Gherezghiher, T. and M.C. Koss, 1979. Clonidine mydriasis in the rat. Eur. J. Pharmacol., 57: 263-266.

Guyenet, P.G., 1997. Is the hypotensive effect of clonidine and related drugs due to imidazoline binding sites? Am. J. Physiol.-Regul. Integr. Comp. Physiol., 273: R1580-R1584.

Koss, M.C. and L.C. San, 1976. Analysis of clonidine-induced mydriasis. Invest. Ophthalmol. Visual Sci., 15: 566-570.

Koss, M.C., 1986. Pupillary dilation as an index of central nervous system $\alpha_2$-adrenoceptor activation. J. Pharmacol. Methods, 15: 1-19.

Koss, M.C., 2003. Rilmenidine produces mydriasis in cats by stimulation of CNS $\alpha_2$-adrenoceptors. Autonomic Autacoid Pharmacol., 23: 51-56.

Nasal, A., T. Frackowiak, J. Petrusewicz, A. Bucinski and R. Kaliszan, 1995. Mydriasis elicited by imidazol(in)e $\alpha_2$-adrenomimetics in comparison with other adrenoceptor-mediated effects and hydrophobicity. Eur. J. Pharmacol., 274: 125-132.

Stabile, A.M., H. Aceros, K. Stockmeyer, A.A.A. Rahman, N. Noiseux and S. Mukaddam-Daher, 2011. Functional and molecular effects of imidazoline receptor activation in heart failure. Life Sci., 88: 493-503.

Szabadi, E. and C.M. Bradshaw, 1996. Autonomic pharmacology of $\alpha_2$-adrenoceptors. J. Psychopharmacol., 3: 6-18.

Szabo, B., T. Fritz and K. Wedzony, 2001. Effects of imidazoline antihypertensive drugs on sympathetic tone and noradrenaline release in the prefrontal cortex. Br. J. Pharmacol., 134: 295-304.

Tolentino-Silva, F.P., M.A. Haxhiu, S. Waldbaum, I. Dreshaj and P. Ernsberger, 2000. $\alpha_2$-adrenergic receptors are not required for central anti-hypertensive action of moxonidine in mice. Brain Res., 862: 26-35.

Van Zwieten, P.A., M.J. Thoolen and P.B. Timmermans, 1984. The hypotensive activity and side effects of methyldopa, clonidine and guanfacine. Hypertension, 6: 28-33.

Walland, A. and W. Kobinger, 1971. On the problem of tolerance of 2-(2, 6-dichlorophenylamino)-2-imidazoline. Arzneimittel-Forschung, 21: 61-65.

Yu, Y. and M.C. Koss, 2005. Rat clonidine mydriasis model: Imidazoline receptors are not involved. Autonomic Neurosci., 117: 17-24.