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Are Alpha-2D Adrenoceptor Subtypes Involved in Rat Mydriasis Evoked by New Imidazoline Derivatives: Marsanidine and 7-Methylmarsanidine?

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Abstract

The imidazoline compounds may produce mydriasis after systemic administration to some species (rats, cats, and mice). In mydriatic activity of imidazolines, α_{2D}-adrenoceptors subtype(s) seems to be involved. In this study, the pupil dilatory effect evoked by 2 newly synthesized imidazoline derivatives—α_2-adrenoceptor agonists: marsanidine and 7-methylmarsanidine—was compared. The compounds were tested alone as well as in the presence of α_2-adrenoceptor antagonists (nonselective, yohimbine, and selective toward the following α_2-adrenoceptor subtypes—α_{2A}-2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3- dihydro-1-methyl-1H-isoindole maleate (BRL44408), α_{2B}-2-[2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione dihydrochloride (ARC239), α_{2C}-JP1302, α_{2D}-2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole hydrochloride [RX821002]). The agonists were studied in male Wistar rats and were administered intravenously in cumulative doses. The antagonistic compounds were given in a single dose before the experiment with marsanidine or 7-methylmarsanidine. Pupil diameter was measured with stereoscopic microscope equipped in green light filter. Marsanidine and 7-methylmarsanidine exerted marked mydriatic effects. BRL44408, JP1302, and ARC239 did not cause significant parallel shift to the right of the dose–effect curves obtained for both imidazolines. In case of yohimbine and RX821002, the marked parallel shifts of dose–response curves were observed, with the antagonistic effects of RX821002 more pronounced. In vivo pharmacodynamics experiment suggests that α_{2D}-adrenoceptor subtype is mainly engaged in mydriatic effects evoked in rats by imidazoline derivatives, in particular by clonidine.

Keywords

rat eye mydriasis, α_2-adrenoceptors subtypes, α_2-adrenergic imidazoline agents, marsanidine, 7-methylmarsanidine, clonidine

Introduction

The compounds having imidazol(in)e moiety (the so-called "clonidine-like" agents) are showing a variety of pharmacological activities, such as hypotension, bradycardia, sedation, analgesia, and mydriasis.[1,2] These effects can be explained by the affinity of imidazol(in)es to the α-adrenergic and imidazoline receptors. It has been proved that α_2-adrenergic agonists of imidazol(in)e structure evoke mydriasis in laboratory animals (rats, mice, and cats) after systemic application.[3,4] Further studies lead to the conclusion that pupillary dilation produced by these compounds is mediated via the stimulation of the brain α_2-adrenoceptors located in the Edinger-Westphal nucleus where they inhibit parasympathetic tone to the iris.[5,6] Christensen et al[7] and Hey et al[5] demonstrated in experiments on both anesthetized and conscious rats that these α_2-adrenergic receptors are located postsynaptically to noradrenergic neurons.[8]

Based on molecular biological and radioligand receptor binding techniques, α_2-adrenoceptors are divided into 4

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subtypes—α_{2A}, α_{2B}, α_{2C}, and α_{2D}—[9,10] which are responsible for different physiological processes. The α_{2A} subtype has been identified at first in human platelets and in rabbit spleen.[11,12] The α_{2B} subtype was found in rat tissues (lung and kidney)[11,13] and the α_{2C} subtype in the opossum kidney cell line.[14] The α_{2D} subtype was reported in rat submaxillary gland and in bovine pineal gland.[12,15] However, α_{2A} and α_{2D} receptors have very similar structure and expectingly are the species orthologs.[16] It was suggested that α_{2A} subtype is present in human and pig, while α_{2D}-adrenoceptors is present in rat, mouse, guinea pig, and cow.[17,18] According to Lanier et al[19] and Link et al, rodent α_{2A} subtype was defined as α_{2D} on the basis of very similar structure and ligand binding profile.[16] However, it was also found that the residue in the position 201 in the human species seems to be an important feature that differentiates α_{2A} from α_{2D} pharmacology.[16,17] In addition, some authors underline the lower affinity of yohimbine for the α2D subtype adrenoceptor.[17]

The question arises which subtype(s) of postsynaptic brain α_2-adrenergic receptors could be engaged in mydriatic activity of imidazoline compounds. Based on the results of both radioligand binding and functional studies, it has been postulated by Heal et al[8] that postsynaptic α_2-adrenergic receptors, localized in the rat cortex and Edinger-Westphal nucleus, are predominantly of α_{2D} subtype.

The aim of the present study was an in vivo assessment in anesthetized rats of the effects on pupil diameter due to marsanidine and 7-methylmarsanidine—2 newly synthesized α_2-adrenergic receptor agonists having an imidazoline moiety in their structure.[18] The well-established mydriasis model according to Koss[3] was applied. The activity of marsanidine and 7-methylmarsanidine was compared to clonidine, a reference imidazoline drug stimulating brain α_2-adrenoceptors. All agents were studied as administrated alone and after the pretreatment with yohimbine—a "classical" nonselective antagonist of α_2-adrenoceptors. To test pharmacologically, whether the α_{2D}-adrenergic receptor is (or not) solely involved in mydriatic effects of marsanidine and 7-methylmarsanidine, the separate experiments were carried out in the presence of the known selective antagonists of individual α_2-adrenoceptor subtypes—2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole maleate (BRL44408; α_{2A}),[20] 2-[2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione dihydrochloride (ARC239; α_{2B}),[11] and JP1302 (α_{2C}).[21] Additionally, 2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole hydrochloride (RX821002), classified as a compound preferentially blocking α_{2D}-adrenoceptors, was included in the project.[22]

Aim

The aim of the study was the comparison of the pupil dilatory effects evoked in rats by a model imidazoline drug—clonidine—and 2 newly synthesized imidazoline compounds: marsanidine and 7-methylmarsanidine. The compounds were tested alone and also in the presence of α2-adrenoceptor antagonists. The aim of the study was also the pharmacological evaluation of the role of α2-adrenergic receptor subtype(s) in mydriatic effects evoked by compounds studied using α2-adrenergic receptor antagonists.

Materials and Methods

Animals

The studies were performed in male Wistar rats weighing 200 to 300 g. The rats were anesthetized with urethane 1.5 g/kg intraperitoneally.

This study was carried out in accordance with the recommendations of "National ethics committee for animal researches in Poland." The protocol was approved by the "National ethics committee for animal researches in Poland."

Imidazoline Derivatives

Clonidine hydrochloride, BRL44408 maleate, JP1302 dihydrochloride, ARC239 dihydrochloride, and RX821002 hydrochloride were purchased from Tocris (Bristol, United Kingdom). Yohimbine hydrochloride was obtained from Sigma-Aldrich (St Louis, Missouri); marsanidine and 7-methylmarsanidine hydrochlorides were synthesized by Prof F. Sączewski at the Department of Chemical Technology of Drugs, Medical University of Gdańsk. All substances studied were dissolved in 0.9% NaCl solution.

Rat Eye Pupil Diameter Measurement

Pupil diameter measurement was carried on by adapting the Koss method.[3] Measurements were performed 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 eliminate the reaction of the pupil on the light and also to enhance the image contrast of the iris. All experiments were performed in a darkened room at fixed light conditions. Pupil diameter was measured with an accuracy of 0.10 mm at the maximum width of the pupil. The initial value of the pupil diameter, before the administration of 0.9% NaCl solution and studied drugs, was about 0.70 ± 0.5 mm.

Clonidine, marsanidine, and 7-methylmarsanidine were administered to the rats through the femoral vein at a volume of 1 mL/kg in cumulative doses (1, 3, 5, 10, 30, 50, 100, 300, 500, 1000 μg/kg) at 5-minute intervals. Yohimbine (1.5 mg/kg), BRL44408 (1 mg/kg), JP1302 (1 mg/kg), ARC239 (0.5 mg/kg), and RX821002 (0.05 mg/kg) were given intravenously 10 minutes before starting the administration of a series of agonist in cumulative doses.

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) of clonidine, marsanidine, and 7-methylmarsanidine, given to the animals alone and in the presence of antagonists.

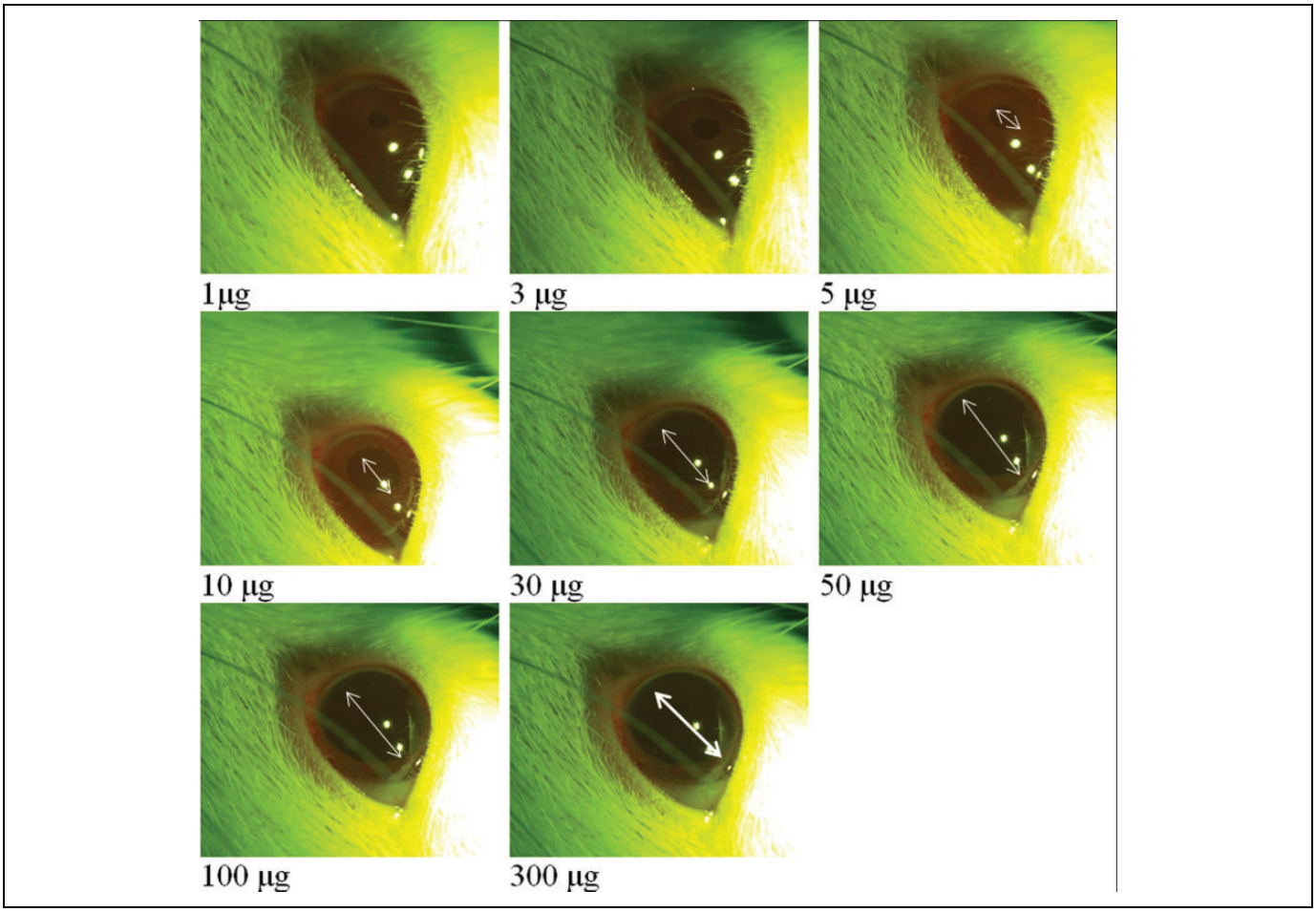

Figure 1. Changes in pupil diameter of the rats after administration of marsanidine in cumulative doses.

Data Analysis

Dose–mydriatic effect curves were constructed applying GraphPad Prism, version 6.00 for Windows, GraphPad Software (La Jolla, California). The doses of imidazoline agents studied, which produced 50% maximum mydriatic effect, the dose causing 50% of maximum effect (ED_{50}), were also calculated by nonlinear regression analysis with the use of this program. These data were presented with 95% confidence intervals and the number of degrees of freedom (*df*) given in parentheses.

Antagonistic potencies of yohimbine, BRL44408, ARC239, JP1302, and RX821002 were expressed as a pA_2 defined as the negative logarithm to base 10 of the molar concentration of an antagonist that makes it necessary to double the concentration of the agonist needed to elicit the original submaximal response obtained in the absence of antagonist.[23] GraphPad Prism was used for calculation of pA_2 values applying Gaddum-Schild model with 95% confidence intervals and the number of *df*. Molecular dynamics calculations[24] were applied when attempting to identify molecular descriptors of imidazolines determining interactions with hypothetical receptors. Unfortunately, our efforts failed.[24] One-way analysis of variance was used to compare the mydriatic effect among the 6 groups (the clonidine and clonidine with each of 5 antagonists studied). Post hoc Tukey test was performed to compare the difference of effects between the groups at a significance level of $P < .05$.

Results

Intravenous administration of clonidine, marsanidine, and 7-methylmarsanidine in increasing doses 1 to 1000 μg/kg resulted in sigmoid mydriatic dose–response curves (Figures 2–4). The pupil dilation was rapid in onset within the first minute after injection to rats and was sustained for the duration of the experiment. The rank order of potency of the imidazoline agents studied was 7-methylmarsanidine > clonidine > marsanidine (Table 1). Maximal pupillary dilations observed, E_{max}, were 3.52 ± 0.10, 3.63 ± 0.09, and 3.97 ± 0.10, respectively.

When rats were pretreated with α_2-adrenoceptor antagonist: yohimbine, BRL44408, ARC239, JP1302, and RX821002, dose-dependent pupillary dilation curves observed for clonidine, marsanidine, and 7-methylmarsanidine were shifted in parallel fashion to the right. The maximal mydriatic responses of the agents under study were indistinguishable from the effects of these imidazoline compounds alone, indicating competitive antagonism (Figures 2–4). The corresponding ED_{50} and pA_2 values are collected in Table 1.

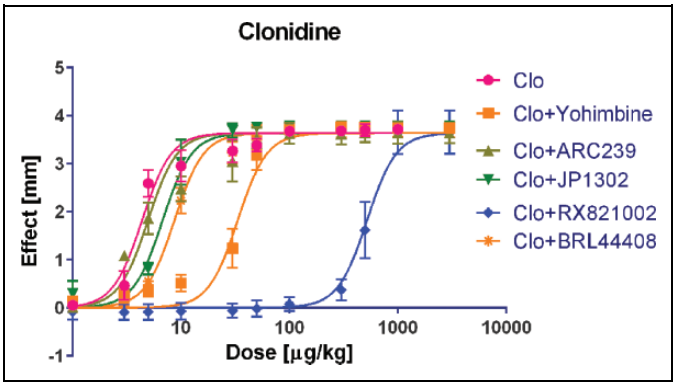

Figure 2. Comparison of the mydriatic effect evoked by clonidine alone and in the presence of α2-adrenoceptor antagonists.

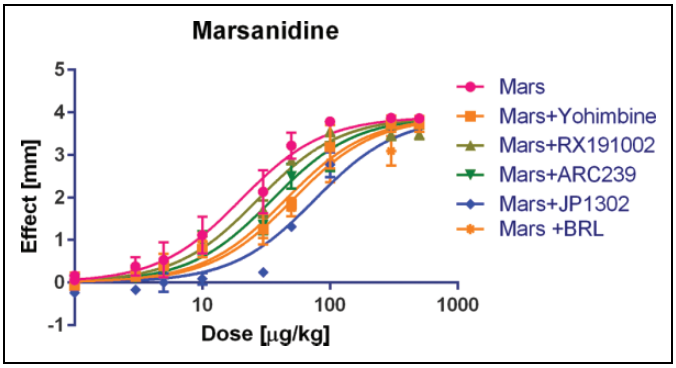

Figure 3. Comparison of the mydriatic effect evoked by marsanidine alone and in the presence of α2-adrenoceptor antagonists.

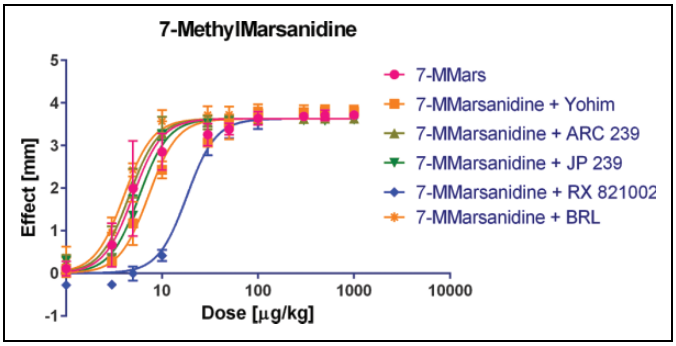

Figure 4. Comparison of the mydriatic effect evoked by 7-methylmarsanidine alone and in the presence of α2-adrenoceptor antagonists.

As shown in Figure 1, RX821002, the preferential antagonist of α_{2D}-adrenergic receptor, is the most potent inhibitor of clonidine-induced mydriasis in rat model. This compound causes a parallel shift to the right of the dose–mydriatic effect curve for clonidine. The calculated ED_{50} values are 524.1 (485.0-566.4) µg/kg and 11.23 (11.01-11.46), respectively (Table 1). Also, the pupillary response curve for clonidine was competitively antagonized by yohimbine in a dose-related fashion, but this effect was less pronounced. The calculated ED_{50} for clonidine + yohimbine is 34.79 (32.89-36.80) and pA_2 value equals 6.66 (6.54-6.79; Table 1). BRL44408, ARC239, and JP1302—the selective antagonists of α_{2A}, α_{2B}, and α_{2C} subtypes of α_2-adrenoceptor, respectively—have no significant effects on mydriasis produced by cumulative doses of clonidine. The corresponding ED_{50} values for clonidine pretreated with BRL44408, ARC239, and JP1302 are close to ED_{50} value for clonidine alone (Table 1). The antagonistic potencies (pA_2) could not be calculated because of the overlapping curves of the dose–mydriatic effects, obtained for clonidine in the presence of BRL44408, ARC239, and JP1302 (Figure 1).

In case of 7-methylmarsanidine, similar situation occurred (Figure 2). Mydriatic effect produced by cumulative doses of this imidazoline agent was strongly antagonized by pretreatment with RX821002 (ED_{50} = 18.11 [16.44-19.94], pA_2 = 6.99 [6.81-7.17]) and less when yohimbine was used (ED_{50} = 6.54 [5.89-7.24], pA_2 = 5.66 [5.41-5.92]; Table 1). BRL44408, ARC239, and JP1302 had no significant effects on pupillary dilation evoked by 7-methylmarsanidine. The ED_{50} values obtained for 7-methylmarsanidine in the presence of BRL44408, ARC239, and JP1302 are close to the value for 7-methylmarsanidine alone (Table 1). Similarly as in the case of clonidine, it was impossible to calculate the values of pA_2 for 7-methylmarsanidine studied in the presence of BRL44408, ARC239, and JP1302 due to the overlapping of dose–pupillary dilation effect curves (Figure 1).

Pretreatment with RX821002 caused a marked parallel shift to the right of the marsanidine mydriasis curve (Figure 3). The potency of yohimbine to inhibit marsanidine-induced mydriasis is lower. The corresponding pA_2 values are 8.34 (8.18-8.49) and 6.02 (5.79-6.24), respectively (Table 1). The antagonists of α_2-adrenoceptor subtypes, ARC239 (α_{2B}) and JP1302 (α_{2C}), produced slight parallel shifts to the right of the marsanidine pupillary dilation curves. The antagonistic potencies (pA_2) of these compounds do not statistically differ as compared to pA_2 value calculated for marsanidine + yohimbine. BRL44408 practically did not shift the marsanidine dose–response curve; therefore, its pA_2 value was not calculated (Table 1).

Discussion

Imidazol(in)e agents, classified as α_2-adrenoceptors ligands, may interact with different subtypes of this receptor (α_{2A}, α_{2B}, α_{2C}, and α_{2D} subtype). However, the role of individual α_2-adrenergic receptor subtypes in physiological processes is still not satisfactorily elucidated. As it was demonstrated in studies of many researchers, adrenoceptors of α_{2A} subtype could play a significant role in hypotension and bradycardia[25,26] as well as in antinociceptive activity.[27,28] This receptor subtype seems to be responsible also for sedative and hypothermic effects.[27] It is also postulated that α_{2A}-adrenoceptors take part in presynaptic inhibition of noradrenaline release in nerve endings at high stimulation frequencies (while release of this neurotransmitter on lower frequencies is regulated rather by α_{2C}-adrenoceptors).[29]

Table 1. ED_{50} Values of Imidazoline Agents Studied in the Absence and in the Presence of Different α_2-Adrenoceptor Antagonists as well as pA_2 Values Calculated for Clonidine, Marsanidine, and 7-Methylmarsanidine in the Presence of Yohimbine, BRL44408, ARC239, JP1302, and RX821002.[a]

Compound	ED_{50}, μg/kg	pA_2
Clonidine	8.34 (7.55-9.18), *df* = 52	–
Clonidine + yohimbine	34.79 (32.89-36.80), *df* = 52	6.66 (6.54-6.79), *df* = 77
Clonidine + BRL44408	8.75 (8.17-9.38), *df* = 41	NC
Clonidine + ARC239	5.56 (4.88-6.32), *df* = 52	NC
Clonidine + JP1302	6.93 (6.58-7.30), *df* = 52	NC
Clonidine + RX821002	524.1 (485.0-566.4), *df* = 47	11.23 (11.01-11.46), *df* = 97
Marsanidine	45.65 (39.60-52.63), *df* = 47	–
Marsanidine + yohimbine	109.9 (84.2-143.4), *df* = 47	6.02 (5.79-6.24), *df* = 77
Marsanidine + BRL44408	114.3 (89.38-146.1), *df* = 33	NC
Marsanidine + ARC239	109.0 (71.93-165.2), *df* = 27	6.55 (6.23-6.87), *df* = 57
Marsanidine + JP1302	68.2 (55.98-83.08), *df* = 47	NC
Marsanidine + RX 821002	153.4 (131.1-179.6), *df* = 37	8.34 (8.18-8.49), *df* = 67
7-Methylmarsanidine	4.94 (4.28-5.93), *df* = 42	
7-Methylmarsanidine + yohimbine	6.54 (5.89-7.24), *df* = 37	5.66 (5.41-5.92), *df* = 87
7-Methylmarsanidine + BRL44408	4.14 (3.89-4.42), *df* = 37	NC
7-Methylmarsanidine + ARC239	4.5 (4.21-4.81), *df* = 37	NC
7-Methylmarsanidine + JP1302	5.65 (5.33-5.99), *df* = 37	NC
7-Methylmarsanidine + RX821002	18.11 (16.44-19.94), *df* = 47	6.99 (6.81-7.17), *df* = 97

Abbreviations: ARC239, 2-[2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione dihydrochloride; BRL44408, 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole maleate; *df*, degrees of freedom; IV, intravenous; JP 1302, N-[4-(4-methyl-1-piperazinyl)phenyl]-9-acridinamine dihydrochloride; NC, not calculated; RX821002, 2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole hydrochloride.

[a]Agonists (clonidine, marsanidine, 7-methylmarsanidine) were administered IV in increasingly cumulative doses at 5-minute intervals. Antagonists were administered IV 10 minutes before starting the administration of the series of agonist doses. ED_{50} values were calculated by nonlinear regression analysis with 95% confidence intervals and the number of *df*.

Stimulation of α_{2B}-adrenoceptor subtype in vascular smooth muscle evokes increase of blood pressure and counteracts the hypotensive effect of α_{2A}-receptors stimulation in the central nervous system.[30] The adrenoceptors of α_{2C} subtype are located mainly in the central nervous system and could be involved (beyond α_{2A}-receptors) in the regulation of transmitter release.[29] Although their role in hemodynamics is still not fully understood, α_{2C}-adrenoceptors seem to mediate venous vasoconstriction[31] and also may mediate other peripheral actions, for example, play a role in hypothermic effect, secondary to the prominent role of the α_{2A}-subtype.[32]

Commonly applied in vitro radioligand binding method to study an affinity of newly synthesized imidazoline analogs of potential pharmacological activity to α_2-adrenergic/imidazoline receptors is obviously not sufficient to decide whether the agent studied could be regarded as receptor agonist or antagonist. To obtain actual information about the pharmacological properties of imidazoline ligands, the in vivo tests are necessary. The rat eye mydriasis model[3] has many advantages as compared to the other pharmacological tests (eg, clonidine-induced reduction of motor activity in mice[33] in allowing to evaluate the interactions of potential imidazoline ligands with brain α_2-adrenergic receptors). The most important is that the pharmacodynamics experiment could be performed in vivo in a whole animal, with an individual imidazoline compound injected in a wide range of doses (from few μg/kg to several mg/kg). This method is simple and reproducible. Moreover, it provides an opportunity to test not only both the α_2-agonistic and α_2-antagonistic properties of imidazolines studied but also to exclude potential ability of the ligands to interact with I_1-imidazoline receptors because this type of receptor is not practically involved in the mediation of central mydriasis in rats.[34,35]

Marsanidine (1-[(imidazolidin-2-yl)imino]indazole) and 7-methylmarsanidine (1-[imida-zolidin-2-yl)imino]-7-methylindazole) are new imidazoline derivatives synthesized by Sączewski et al.[36] In radioligand studies performed on rat brain membranes, the first one proved to be a selective α_2-adrenoceptor ligand (K_i = 14.05 nM) having the α_2/I_1 selectivity ratio 3879, while the second compound shows less affinity to α_2-adrenergic receptor (K_i = 53.6 nM) and its α_2/I_1 selectivity ratio equals 7.2.[37] Both agents exert agonistic activity toward α_2-adrenoceptors, lowering blood pressure and decreasing heart rate in experiments on anesthetized rats.[37,38]

The central antihypertensive agent clonidine shows "mixed" agonistic properties toward α_2-adrenergic and I_1-imidazoline receptors. Its radioligand binding affinity, pK_i values at the human α_{2A}- and α_{2B}-receptors expressed in human embryonic kidney 293 cells are 7.21 and 7.16, respectively, while the corresponding pK_i I_1 value determined in vitro in human platelets equals 7.25. Clonidine injected intravenously to the rats evokes a dose-dependent pupil dilation at very low doses (from 1 μg/kg). Hence, this drug proved to be a good reference agent for other imidazolines having affinity to α_2-adrenergic receptors.[3]

In the present study, 2 newly synthesized imidazoline derivatives—marsanidine and 7-methylmarsanidine—as well as the reference drug: clonidine—produced dose-related mydriatic effects. The maximal pupillary dilations observed after administration of these agents to rats were similar but the effect of marsanidine was slightly greater than in the case of clonidine. It can be noted that the ED_{50} value for clonidine (8.34 μg/kg) is close to that previously reported by Koss[6] and Yu and Koss.[34]

Of the 2 new imidazolines, the relatively selective α_2-adrenergic receptor agonist marsanidine displayed about 5-fold lower potency than clonidine, while the potency of 7-methylmarsanidine ($ED_{50} = 4.94$ μg/kg), having α_2/imidazoline I_1 receptor agonistic properties, is comparable to clonidine. High central activity of these compounds could be connected with their physicochemical properties. The theoretically calculated by us with the use of ACD software[39] lipophilicity parameter, Calculated LOGP, for 7-methylmarsanidine equals 1.70 and is greater than for the 2 remaining imidazolines: clonidine (1.41) and marsanidine (1.24). According to Sączewski et al,[37] clonidine having pK_a value 8.2 is ionized at physiological pH (14% of nonionized form at pH 7.4), whereas marsanidine and its 7-methyl analogue are characterized by lower basicity (pK_a values of these compounds are 6.32 and 6.53, respectively). Therefore, at physiological pH, it is very likely that marsanidine and 7-methylmarsanidine could exist primarily as nonionized bases (92% and 82%, respectively). That would explain their increased ability to permeate the blood–brain barrier. Boblewski et al[38] proved that marsanidine and its 7-methyl derivative administered in the dose of 100 μg/kg to anesthetized rats induced marked decrease of blood pressure and heart rate, but the maximum hypotensive and negative chronotropic effects of the former compound (−30 mm Hg and −49 beats per minute) were less pronounced than that of the second one (−43 mm Hg and −122.9 beats per minute).

Yohimbine is an α_2-adrenergic antagonist commonly used in studies on the mydriatic activity of imidazoline compounds. In radioligand studies, it binds to all α_2-adrenoceptor subtypes, having the higher affinity to the α_{2A} and α_{2C} subtypes, lower to α_{2B} one, and the lowest to α_{2D}-adrenergic receptor. The corresponding pK_i values determined with the use of membranes of tissues containing only 1 subtype of α_2-adrenergic receptor—HT29 cells (α_{2A}), rat neonatal lung (α_{2B}), opossum kidney cells (α_{2C}), and PC12 cells (α_{2D})—are 8.72, 7.95, 8.94, and 7.27, respectively.

The another 3 α_2-adrenergic receptor antagonists, applied in this work, are characterized by a selectivity toward particular subtypes of this receptor. BRL44408 is classified as selective α_{2A}-adrenoceptor antagonist having a good affinity for this subtype but significantly lower for all other subtypes of α_2-adrenergic receptor. In radioligand binding studies on Chinese hamster ovary (CHO) cells, transfected with human α_{2A}, α_{2B}, and α_{2C} receptors, K_i values were 109, 1800, and 700 nM, respectively.[20]

ARC239 exerted a slight preference for α_{2A}-adrenoceptors and showed significant α_{2B}-adrenoceptor selectivity displaying a 100-fold α_{2B}/α_{2A} selectivity ratio in cell line experiments. Its binding affinity values, pK_i, in CHO cell lines expressing human α_{2A}, $\alpha_{2B,}$ and α_{2C} adrenergic receptors were 6.65, 8.03, and 7.78, respectively.[40]

JP-1302 is a novel highly specific α_{2C}-adrenoceptor ligand. In in vitro competition binding assays with [^{3}H]-rauwolscine, on membranes from S115 cells transfected with 1 of the 3 human α_2 receptor subtypes (α_{2A}, α_{2B}, α_{2C}), the agent displayed an affinity of 28 nM for the α_{2C} subtype. The same K_i values obtained for the α_{2A}- and α_{2B}-adrenergic receptors are 3150 and 1470 nM, respectively.[41] JP-1302 displayed strong antagonistic potency, characterized by K_B value of 16 nM, at the human α_{2C}-adrenoceptor subtype. In comparison, the K_B for human α_{2A} and α_{2B} subtypes equals 1500 and 2200 nM, respectively. All these data were established with membranes from CHO cells, stably expressing the human α_{2A}, α_{2B}, and α_{2C} adrenergic receptor subtypes, by antagonizing the adrenaline-induced stimulation of [^{35}S]-GTPγ binding.[41]

According to Sallinen et al, JP-1302 did not antagonize dexmedetomidine-evoked mydriatic effect in rats, but this effect was antagonized by atipamezole known as a nonselective antagonist of α_2-adrenoceptor subtypes.[41]

RX821002 is relatively selective for both α_{2A} and α_{2C} versus α_{2B} adrenoceptor subtypes. Its binding activity, pK_i, at 3 human α_2-adrenergic receptor subtypes expressed in CHO cells, is 9.73 (α_{2A}), 8.77 (α_{2B}), and 9.52 (α_{2C}), respectively.[42] At the same time, in experiments on brain cortex slices, this compound is an antagonist with high power to distinguish α_{2A} from α_{2D}-adrenoceptors while having markedly higher affinity for guinea pig α_{2D} ($pK_d = 9.7$) than rabbit α_{2A} ($pK_d = 8.2$) subtypes.[43]

Dose–pupillary dilation curves, obtained not only for clonidine but also for marsanidine and 7-methylmarsanidine, were parallelly shifted to the right by yohimbine, which supports the participation of brain α_2-adrenergic receptors in mydriatic action of a model compound and 2 new imidazoline derivatives. Analysis of variance ($P = .02$) and Tukey analysis of the results of our further experiments with the use of RX821002 showed that in the case of clonidine the subtype α_{2D} seems to be predominantly engaged in pupillary response evoked by the imidazolines studied. The results of our further experiments with the use of BRL44408, ARC239, JP-1302, and RX821002 showed that the subtype α_{2D} is predominantly engaged in pupillary response evoked by imidazolines studied. It was demonstrated by marked changes of pA_2 values for clonidine, marsanidine, and 7-methylmarsanidine pretreated with RX821002, as compared to corresponding pA_2 values calculated for these agents studied at the presence of yohimbine. Whereas in the case when selective antagonists of α_{2A}, α_{2B}, α_{2C} subtypes of α_2-adrenoceptor were administered in single doses prior to clonidine, marsanidine, and 7-methylmarsanidine, no changes in the mutual position of the corresponding dose–response curves were noted.

Previously Heal et al[44] provided the data from experiments in vitro on rat brain cortex preparation using a series of ligands

having different affinity to particular α_2-adrenergic receptor subtypes (α_{2A}-α_{2D}). Displacement of [^{3}H]RX821002 from cortical membranes with these compounds yielded pK_i values correlated very well with the same values for the α_{2D} receptors in rat submaxillary gland reported earlier by Michel et al[12] At the same time, no significant correlations were obtained with literature pK_i data characterizing the binding of the agents to α_{2A}, α_{2B}, and α_{2C} subtypes localized in rabbit spleen (α_{2A}), rat kidney (α_{2B}), and OK cells (α_{2C}).[44] Heal et al[44] determined also in conscious rats the potencies of various α_2-adrenoceptor antagonists to inhibit clonidine-evoked mydriasis and found good relationships between $-\log ID_{50}$ values and pK_i values for α_{2D}-adrenoceptor binding, whereas poor correlations with the K_i for the remaining subtypes of these receptors were noted. Based on the presented data, the conclusion has been drawn by these authors that postsynaptic α_2-adrenergic receptors localized in both brain cortex and the Edinger-Westphal nucleus of the rat could be mainly of the α_{2D} subtype.

However, lack of highly selective antagonists of particular subtypes of α_2-adrenoceptors was for a long time an obstacle to demonstrate directly which subtype is engaged in mydriatic activity of imidazoline agents in rats. Especially, it concerns the following receptors: α_{2A}, α_{2B}, and α_{2C}. Nowadays, some of these antagonists are available, for example, BRL4408 (α_{2A}), ARC239 (α_{2B}), JP-1302 (α_{2C}), but according to existing literature, they were not yet applied (except of JP-1302)[41] in functional studies on rat eye mydriasis. Only some antagonists, such as RX821002, MK 912, and benoxathian, are known to unambiguously differentiate α_{2A} from α_{2D}-adrenoceptors.[45] Therefore, in this work, we undertook an attempt to evaluate directly the involvement of α_{2D}-adrenoceptors in pupillary dilation produced by imidazoline drugs in anesthetized rats. Our results seem to confirm the earlier observations by Heal et al.[44] Moreover, results support observations by Yu and Koss[34] that clonidine-evoked mydriasis is triggered by postsynaptic α_2-adrenergic stimulation of sciatic nerve, which produced mydriasis by the reduction of parasympathetic neural tone to the iris. Presented results (especially as regards to marsanidine and 7-methylmarsanidine) indicated that rat eye mydriasis model seems to be a valuable tool not only for detailed studies on the mechanism of imidazolines action on brain α_2-adrenoceptors but also for identification of potential cardiovascular and other (eg, antinociceptive, antidepressant, anesthetic) drug "candidates" among newly synthesized imidazoline derivatives.

Conclusion

New imidazoline compounds, marsanidine and 7-methylmarsanidine show strong mydriatic effects in rats as compared to clonidine. Experiments performed in the presence of α_2-adrenoceptor subtype(s) antagonists seem to confirm that α_2-adrenoceptor 2D subtype has been engaged in mydriatic effects of clonidine, but results for marsanidine and 7-methylmarsanidine are ambiguous.

Declaration of Conflicting Interests

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