Atypical sympathomimetic drug lerimazoline mediates contractile effects in rat aorta predominantly by 5-HT2A receptors

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ABSTRACT

Lerimazoline is a sympathomimetic drug that belongs to the imidazoline class of compounds, and is used as a nasal decongestant. Studies on lerimazoline are rare, and its pharmacological profile is not completely understood. Here, we analyzed the affinity of lerimazoline for dopamine receptor D2, serotonin 5-HT1A and 5-HT2A receptors and α1-adrenoceptor, and investigated lerimazoline contractile effects in isolated rat thoracic aorta. We also determined the effect of several antagonists on the contractile response to lerimazoline, including prazosin (α1-adrenoceptor antagonist), RX 821002 and rauwolscine (α2-adrenoceptor antagonists), methiothepin (non-selective 5-HT receptor antagonist), SB 224289 (5-HT1D receptor antagonist), BRL 15572 (5-HT1D receptor antagonist), and ketanserin (5-HT2A receptor antagonist). Lerimazoline displayed high affinity for the 5-HT1A receptor (Ki = 162.5 nM), similar to the previously reported affinity for the 5-HT1A receptor. Binding affinity estimates (Ki) for α1, 5-HT2A, and D2 receptors were 6656, 4202 and 3437.5 nM, respectively (the literature reported Ki for 5-HT1B receptor is 3480 nM). Lerimazoline caused concentration-dependent contractions in 70% of preparations, varying in the range between 40% and 55% of the maximal contraction elicited by phenylephrine. While prazosin reduced the maximum contractile response to lerimazoline, rauwolscine showed a non-significant trend in reduction of the response. Both ketanserin (10 nM and 1 µM) and methiothepin strongly suppressed the maximum response to lerimazoline. Overall, our results suggest that 5-HT1A and, less distinctly, α1-adrenergic receptors are involved in the lerimazoline-induced contractions, which makes lerimazoline an “atypical” decongestant.

KEY WORDS: Lerimazoline; rat aorta; binding affinity; phenylephrine; 5-HT2A receptors; St-71; trimizoline; tramazoline; antagonist activity; sympathomimetic drug

INTRODUCTION

Several sympathomimetic drugs are used as topical nasal decongestants. These drugs are commonly classified into sympathomimetic amines (e.g., phenylephrine and ephedrine) and imidazolines (e.g., oxymetazoline and xylometazoline). It is assumed that the both classes induce a decongestant effect through the activation of α-adrenoceptors in the nasal vasculature. Topical decongestants are short-term effective in relieving nasal congestion, a prominent symptom associated with conditions such as common cold, allergic rhinitis, sinusitis or otitis media [1]. However, due to their indiscriminate use there is a risk of development of rebound congestion or rhinitis medicamentosa [2]. The risk of such a serious complication may be prevented or at least delayed using a polypharmacological, as opposed to single-target approach, aimed to optimize the therapeutic outcome by concomitant modulation of multiple rather than single drug targets [3,4].

Basic research data suggest that imidazoline decongestants act not only through the α-adrenergic but also through serotonin 5-hydroxytryptamine (5-HT) receptors [5-7]. Thus, both oxymetazoline and xylometazoline have been recognized as high-affinity, high-efficacy 5-HT2A receptor agonists [8]. Although not specifically linked to imidazoline decongestants, activation of these receptors may cause vasoconstricting effects, and is also associated with a risk for drug-induced valvular heart disease [8,9]. On the contrary, the activation of other receptors could be a potential benefi-
a role in the regulation of nasal airway resistance and nasal secretion [10].

Lerimazoline is the international nonproprietary name recently adopted for 2-[(2,4,6-trimethylphenyl)methyl]-4,5-dihydro-1H-imidazole, a congener of oxymetazoline and xylometazoline, originally patented in 1963 [11,12]. In combination with phenylephrine, this sympathomimetic drug of the imidazole class has been used as a nasal decongestant for decades in several countries, in the concentration of 2.1 × 10^{-4} M for children and 6.3 × 10^{-4} M for adults [13]. However, the primary research studies on this compound, reported earlier as St-71, trimizolone or trimazoline, are scarce [14-17] and its pharmacological profile is not completely understood. While lerimazoline has a substantial affinity for the 5-HT_2A receptor and much lower affinity for 5-HT_1B receptors (Ki values 72 versus 3480 nM) [6], its affinity for other 5-HT or α-adrenergic receptors has not been specifically examined. Importantly, Law et al. [6] evaluated lerimazoline for contractile activity in the rings of rabbit saphenous vein and found that it induces contractions in an α-adrenoceptor- and prostan glandin-independent manner [6].

Here, we analyzed the affinity of lerimazoline for the dopamine receptor D_2, 5-HT_1A, and 5-HT_1D receptors and α_1-adrenoceptor, and investigated lerimazoline contractile effects in isolated rat thoracic aorta [18,19]. We also determined the effect of eight antagonists on the contractile response to lerimazoline.

MATERIALS AND METHODS

Membrane preparation, radioligand binding assays, and data analysis

Specific binding affinities were determined as described previously [20] by measuring the extent of displacement of [3H] labelled specific ligands, purchased from Perkin Elmer LAS GmbH, (Rodgau, Germany). A range of concentrations (10^{-4} to 10^{-6} M) of [3H] Spiperone [specific activity (spec. act.) 73.36 Ci mmol^{-1}], 8-Hydroxy-[3H] DPAT (spec. act. 142 Ci mmol^{-1}), [3H] Ketanserin (spec. act. 47.3 Ci mmol^{-1}) and [3H] Prazosin (spec. act. 81.7 Ci mmol^{-1}) were used to label the D_2, 5-HT_1A, 5-HT_1D, and α_1-adrenoceptor, respectively, from rat striatal or cortical synaptosomes. Non-specific binding was measured in the presence of 10 µM of spiperone (D_2), 10 µM of buspirone (5-HT_1A), 10 µM of ketanserin (5-HT_1D), and 10 µM of prazosin (α_1). The tubes (0.4 ml total volume of the incubation mixture for D_2 receptor and 0.2 ml total volume of the incubation mixture for 5HT_1D, 5-HT_1A, and α_1-adrenergic receptors) were incubated for 10 minutes at 37°C, and the reaction was terminated by vacuum filtration through Whatman GF/C filters. The filters were washed two times with 5.0 ml ice-cold incubation buffer. Retained radioactivity was measured by introducing dry filters and 4 ml of toluene-based scintillation liquid and counting in a LKB Wallac 1219 RackBeta Flexi-Vial Spectral Liquid Scintillation Counter (EG&G Wallac, Turku, Finland). Competition binding curves were constructed and analyzed by GraphPad Prism v. 4.0 (GraphPad Software, Inc., San Diego, CA, USA).

Chemicals and drugs

Lerimazoline hydrochloride and phenylephrine hydrochloride were kindly donated from Zdravlje Actavis, Leskovac, Serbia. SB 224289 (5-HT_1A receptor antagonist), BRL 15572 (5-HT_1D receptor antagonist) and RX 821002 hydrochloride (α_1-adrenoceptor antagonist) were purchased from Tocris (Bristol, UK). Ketanserin tartrate (5-HT_1D receptor antagonist), methiothepin hydrochloride (non-selective 5-HT receptor antagonist), prazosin hydrochloride (α_1-adrenoceptor antagonist), rauwolscine hydrochloride (α_2-adrenoceptor antagonist) and JP 1302 hydrochloride (α_3-adrenoceptor antagonist) were purchased from Sigma Aldrich (St. Louis, MO, USA).

All ligands, except SB 224289, BRL 15572 and JP 1302, were prepared as concentrated stock solutions in distilled water before diluting to final volume, and stored at 4°C during the experiment. SB 224289, BRL 15572 and JP 1302 were initially dissolved in 1% dimethyl sulfoxide (DMSO) and subsequent dilutions were carried out in distilled water. Drug concentrations are described as final molar concentrations (mol/liter) in the tissue bath. At the volumes used, none of the vehicles showed pharmacological activity in the blood vessels.

Experimental animals

Adult male Wistar rats weighing 250-400 g were obtained from Military Farm, Belgrade, Serbia. All experimental procedures were approved by the Ethical Commission on Animal Experimentation of the Faculty of Pharmacy in Belgrade and were carried out in accordance with the EEC Directive 86/609. Rats were housed in clean transparent plastic cages with free access to tap water and pelleted food, and kept in a conventional animal facility at a temperature of 22 ± 1°C, relative humidity of 40-70% and the 12 hours light period, with lights on from 06:00.

Preparation of the rat aortic rings

Male Wistar rats were euthanized with carbon dioxide (CO_2) and the thoracic aortas were isolated. Tissues were placed thereafter in Petri dishes containing modified Krebs bicarbonate buffer, cleared of adhering fat and connective tissue, and cut into cylindrical segments with a length of ~2-3 mm. In a number of vessel preparations, the endothelial cell layer was removed by gently rubbing the luminal surface of the ring. The prepared aortic rings were then placed in an organ bath containing 25 ml of Krebs-Ringer bicarbonate...
buffer consisting of 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄·7H₂O, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, and 11 mM glucose, maintained at 37°C, pH 7.4, continuously bubbled with 95% O₂ and 5% CO₂.

Two stainless steel triangles were placed through the lumen of the aortic rings, one was connected to the tissue chamber and the other to the MLT0201 force displacement transducer (Panlab, Spain). The signal was digitized using the Powerlab/ASP data acquisition system (ADInstruments, Castle Hill, Australia) and changes in isometric tension were recorded with LabChart 6 Pro software (AD Instruments).

Segments were allowed to equilibrate for 1 hour in Krebs bicarbonate buffer solution, and the solution was replaced every 10 minutes to prevent the accumulation of metabolic end products. During the next 10 minutes, each aortic ring was stretched progressively to a length optimal for maximal isometric contraction (2 g, as determined in preliminary experiments). Additionally, the rings were allowed to equilibrate for 30-40 minutes before commencing the experiment.

Experimental protocols

In the first series of experiments, endothelial integrity was examined by inducing the contraction of the rat aorta with phenylephrine (10⁻⁴ M) followed by the addition of acetylcholine (10⁻⁵ M). A preparation was considered to have endothelium when the relaxation evoked by acetylcholine was at least 70%. Thereafter, cumulative concentration-response curves (CCRCs) to lerimazoline (concentration range: 10⁻⁷-3 × 10⁻¹ M) were obtained in the rings, previously equilibrated to the basal tone. CCRCs were generated by increasing the concentration of lerimazoline in half-log increments, once the constriction to the previous concentration had stabilized [21,22]. After a wash out period of 30 minutes, the entire procedure has been replicated to confirm that repeated exposure of aortic rings to lerimazoline in the absence of any antagonist evoked similar constrictor responses (data not shown).

In the second series of experiments, each equilibrated rat aorta was first constricted by incremental concentrations of lerimazoline (10⁻⁷-3 × 10⁻¹ M). After a wash out period of 30 minutes during which Krebs-Ringer Bicarbonate Buffer (KRB) buffer was replaced every 10 minutes and the rings returned to the baseline tension, SB 224286 (10⁻⁷ M), BRL 15572 (10⁻⁶ M), ketanserin tartrate (10⁻⁸ and 10⁻⁶ M), prazosin hydrochloride (3 × 10⁻⁷ M), RX 821002 (3 × 10⁻⁸ M), rauwolscine hydrochloride (10⁻⁷ M) or JP 1302 (10⁻⁴ M) were added to the bath and incubated for 20 minutes, before obtaining the CCRCs to lerimazoline in the presence of the respective antagonist. At the end of each experiment, the aortic rings were additionally equilibrated for 30 minutes to the resting tension and finally contracted with phenylephrine (10⁻⁴ M). The strength of constriction was expressed as a percentage of the maximum tension induced by lerimazoline compared to that induced by phenylephrine (10⁻⁴ M). Each segment of the rat aorta was used only for one experimental protocol.

Statistical analysis

Lerimazoline-evoked contractile responses were expressed as mean ± standard error of the mean (SEM) for number (n) of observations, where n equals the number of animals from which aortic rings were isolated. Contraction in each tissue was reported as a percentage of control response to phenylephrine (10⁻⁴ M). The potency of the agonist was measured in terms of EC₅₀ for each curve (the given concentration of lerimazoline was required to produce 50% of the calculated maximum response for the agonist) and presented as pEC₅₀ (the negative log₁₀ of the EC₅₀ value) by using software LabChart 7 (ADInstruments). Antagonist potency was expressed as an apparent pKb value calculated according to the following equation:

\[ pKb = \log(\text{DR} - 1) - \log[B] \]

where DR is the ratio of the mean EC₅₀ value in the presence of the antagonist to the mean EC₅₀ value in the absence of the antagonist; B is the molar concentration of the antagonist tested.

Statistical analysis of CCRCs was performed by unpaired Student’s t Test when comparing two groups, or by analysis of variance (ANOVA) followed by the Student-Newman-Keuls post hoc test in the case of three or more groups. In all cases, a p value less than 0.05 was considered statistically significant. Using SigmaPlot 11 (Systat Software Inc., Richmond, USA) software, a linear regression analysis of published pooled binding data for the analyzed antagonists of α-adrenergic and serotoninergic receptors in humans (or if available in the rat) and our pKb values was conducted [23-25]. At least three pairs of valid data were necessary to generate correlation for a given receptor subtype; if an antagonist acted as a full agonist on a secondary receptor, the data were not included into the analysis.

RESULTS

Binding study

Binding affinities (Ki) of lerimazoline for four examined receptor types (D₂, 5-HT₁A, 5-HT₁D, and α₁-adrenoceptor) are presented in Table 1. Among these receptors, lerimazoline displayed the highest submicromolar affinity for the 5-HT₁A receptor.

Vascular effects of lerimazoline

Lerimazoline caused concentration-dependent contractions of rat aorta rings in approximately 70% of tested
TABLE 1. Binding affinities of lerimazoline to different receptors. For convenience, the published data for lerimazoline at serotonin 5-HT_{1A} and 5-HT_{1B} receptors [6] were also reproduced (designated with an asterisk)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Kᵢ(SEM) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine D_{1}</td>
<td>3437.5±138.9</td>
</tr>
<tr>
<td>Serotonin 5-HT_{1A}</td>
<td>162.5±4.5</td>
</tr>
<tr>
<td>Serotonin 5-HT_{1B}</td>
<td>4202.7±8</td>
</tr>
<tr>
<td>α₁-adrenoceptor</td>
<td>6656±149</td>
</tr>
<tr>
<td>Serotonin 5-HT_{1D}</td>
<td>72±12</td>
</tr>
<tr>
<td>Serotonin 5-HT_{1C}</td>
<td>3480±1690</td>
</tr>
</tbody>
</table>

*Published in Law et al. [6]; SEM: Standard error of the mean

preparations, in the concentration range 3 × 10⁻⁶ – 3 × 10⁻⁴ M; in the rest of aorta preparations, the response was either weak (less than 15% of the maximal effect elicited by phenylephrine) or absent. The results obtained from all 75 aorta preparations (one piece of each aorta from 75 different animals) that responded to lerimazoline, irrespective of the presence of endothelium, are given in Table 2. When vasoactive, lerimazoline produced mean maximal contractions varying in the range between 40% and 55% of the contractions elicited by 10⁻⁴ M phenylephrine (E_{max}; 45.08 ± 1.18% of 10⁻⁴ phenylephrine maximal contraction; pEC_{50}, 4.30, n = 75; the pEC_{50} value for phenylephrine was 5.77). Thus, lerimazoline appear to be a less potent and less effective contractile agent compared to phenylephrine. The effect of lerimazoline was not dependent on the endothelium, i.e., there was no statistical difference between the results obtained in the rat aortas with and without the endothelium (Figure 1). Noticeably, the concentration response curves to lerimazoline showed that the receptors were not saturated at 3 × 10⁻⁴ M. However, a number of experiments using additional concentrations (1 × 10⁻⁴ and 3 × 10⁻³ M) revealed that the concentration response curve to lerimazoline cannot reach the plateau phase (due to a decrease tendency of the contractile response; data not shown) and thus the maximal contraction to lerimazoline was set at 3 × 10⁻⁴ M.

Contractile effects of lerimazoline in the absence and presence of α₁-adrenoceptor antagonists

To examine the involvement of noradrenergic (α₁- and α₂-adrenoceptor) and serotonergic systems in lerimazoline-induced contraction, selective α₁-adrenoceptor and serotonin antagonists were used in the rat aortas (Table 3).

Prazosin (0.3 µM), an α₁-adrenoceptor antagonist, significantly reduced the maximum contractile response to lerimazoline (E_{max}; 44.83 ± 3.28% in the absence versus 27.83 ± 5.32% in the presence of prazosin, p < 0.05, Figure 2A; n = 15). Although prazosin caused a rightward shift in the concentration-response curve to lerimazoline, we were not able to calculate the pKb value because DR was less than 1.

RX 821002 (30 nM, pKb = 8.19), an α₁-adrenoceptor antagonist, did not affect significantly the contractile response to lerimazoline (E_{max}; 40.92 ± 3.65% in the absence versus 35.45 ± 6.31% in the presence of RX 821002, p > 0.05, Figure 2B; n = 15).

An additional set of experiments was carried out with a combination of prazosin (0.3 µM) and RX 821002 (30 nM). The combination of two drugs reduced the maximum response to lerimazoline to similar extent as prazosin (E_{max}; 48.72 ± 4.86% in the absence versus 28.81 ± 6.80% in the presence of prazosin and RX 821002, p < 0.05, Figure 2C; n = 8). As in the former analysis, we were not able to calculate the pKb value (pEC_{50} value for lerimazoline was 3.45 ± 0.16, while for

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<table>
<thead>
<tr>
<th>Drug</th>
<th>pEC_{50}</th>
<th>n</th>
<th>Concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRAZOSIN Untreated</td>
<td>3.93±0.09</td>
<td>15</td>
<td>0.3</td>
</tr>
<tr>
<td>PRAZOSIN Treated</td>
<td>4.01±0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RX 821002 Untreated</td>
<td>3.91±0.26</td>
<td>12</td>
<td>0.03</td>
</tr>
<tr>
<td>RX 821002 Treated</td>
<td>3.15±0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRAZOSIN+RX 821002 Treated</td>
<td>3.45±0.16</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>PRAZOSIN+RX 821002 Untreated</td>
<td>3.51±0.37</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>JP 1302 Untreated</td>
<td>4.07±0.11</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>JP 1302 Treated</td>
<td>3.98±0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAUWOLSCINE Untreated</td>
<td>4.13±0.13</td>
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<td>0.1</td>
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<tr>
<td>RAUWOLSCINE Treated</td>
<td>3.69±0.11</td>
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<td></td>
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<tr>
<td>METHIOTHEPIN Untreated</td>
<td>4.30±0.13</td>
<td>9</td>
<td>0.3</td>
</tr>
<tr>
<td>METHIOTHEPIN Treated</td>
<td>3.99±0.34</td>
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<tr>
<td>BRL 15572 Untreated</td>
<td>3.85±0.14</td>
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<td>1</td>
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<tr>
<td>BRL 15572 Treated</td>
<td>3.77±0.21</td>
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<td></td>
</tr>
<tr>
<td>SB 224289 Untreated</td>
<td>4.24±0.08</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>SB 224289 Treated</td>
<td>3.98±0.08</td>
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<tr>
<td>KETANSERIN Untreated</td>
<td>4.42±0.24</td>
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<td>1</td>
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<tr>
<td>KETANSERIN Treated</td>
<td>3.80±0.27</td>
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<tr>
<td>KETANSERIN Untreated</td>
<td>4.02±0.31</td>
<td>7</td>
<td>0.01</td>
</tr>
<tr>
<td>KETANSERIN Treated</td>
<td>3.89±0.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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Values are expressed as mean±standard error of the mean (SEM); pEC_{50} is the negative log of lerimazoline concentrations inducing 50% of the maximum contractile response.
the combination of prazosin and RX 821002 it was 3.51 ± 0.37; \( p > 0.05 \).

Next, we examined the effect of the nonselective \( \alpha_1 \)-adrenoceptor antagonist rauwolscine and \( \alpha_2 \)-adrenoceptor antagonist JP 1302 on the isolated rat aorta. While JP 1302 (1 µM; pKB = 5.4) did not significantly affect the lerimazoline-induced contraction \( E_{\text{max}}: 37.85 \pm 4.03 \)\% in the absence versus 36.39 ± 5.23\% in the presence of JP 1302 (Figure 3B; \( n = 9 \)), rauwolscine (0.1 µM; pKB = 7.23) caused a rightward shift in the concentration-response curve to lerimazoline, but the result was not significant (38.82 ± 3.98 in the absence versus 27.66 ± 4.14\% in the presence of rauwolscine, \( p = 0.067 \); Figure 3A; \( n = 10 \)).

**Contractile effects of lerimazoline in the absence and presence of serotonergic antagonists**

Methiothepin (a non-selective serotonergic antagonist, 30 nM; pKB = 7.53) strongly suppressed the maximum response to lerimazoline \( E_{\text{max}}: 46.26 \pm 6.06 \)\% in the absence versus 14.58 ± 5.57\% in the presence of methiothepin, \( p < 0.01 \) (Figure 4).

The effects of the selective 5-HT\(_{1B}\), 5-HT\(_{1D}\), and 5-HT\(_{2A}\) antagonists are shown in Figure 5. 5-HT\(_{1B}\) antagonist BRL 15572 did not block the response to lerimazoline at 1 µM (pKB = 5.28; \( E_{\text{max}}: 43.63 \pm 3.58 \)\% in the absence versus 33.73 ± 7.02\% in the presence of BRL 15572, \( p > 0.05 \); Figure 5A; \( n = 12 \)), similarly, the maximum contractile response to lerimazoline was not suppressed by 5-HT\(_{1B}\) antagonist SB 224289 at 1 µM (pKB = 5.92; \( E_{\text{max}}: 38.04 \pm 3.61 \)\% in the absence versus 33.61 ± 6.12\% in the presence of SB 224289, \( p > 0.05 \); Figure 5B; \( n = 10 \)).

Since the rat thoracic aorta is an established model for 5-HT\(_{2A}\) receptor-mediated contraction [26], we examined the effect of ketanserin at two concentrations: 10 nM (pKB = 7.53) and 1 µM (pKB = 6.51). At 1 µM concentration, ketanserin...
significantly inhibited lerimazoline-evoked vasoconstriction [lerimazoline concentrations > 10 µM] (Figure 5C; n = 9); the $E_{\text{max}}$ value was $37.15 \pm 4.66\%$ in the absence versus $19.25 \pm 5.22\%$ in the presence of 1 µM ketanserin, $p < 0.05$. Similar results were obtained for 10 nM concentration of ketanserin (Figure 5D; n = 7); the $E_{\text{max}}$ value was $47.15 \pm 3.14\%$ in the absence versus $19.07 \pm 6.90\%$ in the presence of 10 nM ketanserin, $p < 0.01$.

**Correlation analysis with Ki values from the literature**

Three or more pairs of valid data, necessary to determine linear correlation between Ki (literature data given in Table 4) and calculated pKb values were found for the four receptor subtypes; the analysis for 5-HT$_{1D}$ and 5-HT$_{1A}$ receptors was performed twice, involving separately the pKb values calculated for ketanserin at 10 nM and 1 µM concentration, respectively. A total of 6 correlations were obtained, but only one was statistically significant. For the 5-HT$_{1A}$ receptor a high and significant correlation with the literature values was observed only with the pKb value for 10 nM ketanserin ($r = 0.997$, $n = 3$, $p = 0.0499$), but not with 1 µM of ketanserin ($r = 0.729$, $n = 3$, $p = 0.480$). A significant correlation was not observed for the α$_{2C}$-adrenoceptor ($r = 0.796$, $n = 3$, $p = 0.414$), 5-HT$_{1A}$ receptor ($r = 0.906$, $n = 3$, $p = 0.278$), and 5-HT$_{1D}$ receptor, when both pKb values of ketanserin were included (for 1 µM of ketanserin $r = 0.281$, $n = 5$, $p = 0.646$; for 10 nM of ketanserin $r = 0.257$, $n = 5$, $p = 0.676$).

**DISCUSSION**

Although sympathomimetic properties of lerimazoline are well-known, the exact mechanism of action has been scarcely studied [14-17]. This imidazoline derivative binds with nanomolar affinity to the human 5-HT$_{1D}$ receptors ($K_i = 72$ nM) and with micromolar affinity to the 5-HT$_{1B}$ receptors ($K_i = 3480$ nM) [6]. In cells expressing recombinant 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors lerimazoline markedly suppressed the forskolin-induced increase in cAMP levels, which may be associated with its agonistic effects at both receptor subtypes [6].

Lerimazoline was also evaluated for contractile activity in the rings of rabbit saphenous vein pretreated with prazosin, idazoxan and indomethacin, in order to exclude its possible α$_1$-, α$_2$-adrenergic or prostaglandin-mediated effects, respectively. The EC$_{50}$ of lerimazoline ($150$ nM) was similar to that of the 5-HT$_{1D}$ receptor agonist sumatriptan ($220$ nM) [6]. In another study, treatment with sumatriptan, a 5-HT$_{1D}$ and 5-HT$_{1A}$ receptor antagonist, in rabbit saphenous vein implied that contractile action of lerimazoline on this vein is predominantly associated with the activation of 5-HT$_{1A}$ receptors [27].
However, a relatively low affinity of lerimazoline for the 5-HT<sub>1B</sub> receptors in the study of Law et al. [6] does not correspond with this presumption.

In the present study, we selected rat thoracic aorta as a well-validated and widely used experimental model [18,19]. Lerimazoline caused contractions in the range between 40 and 55% of the maximal contraction elicited by α<sub>1</sub>-adrenoceptor selective antagonist, phenylephrine, in an endothelium-independent manner. The lerimazoline-induced concentration-dependent contractions, found in approximately 70% of tested preparations, could be related to contractile activity of sumatriptan in preparations of some, but not all isolated human mesenteric arteries [28]. In our study, the binding profile of lerimazoline for the α<sub>1</sub>-adrenoceptor and 5-HT<sub>1</sub>A receptors suggests that the 5-HT<sub>1D</sub> and 5-HT<sub>1A</sub> receptors are stimulated first, at concentrations close to 100 nM. However, the contractile action of lerimazoline on the rat aorta started at concentrations close to 10 µM, indicating that some low-affinity binding sites are involved in the contractile response.

In the antagonist studies, we first examined whether the α<sub>1</sub>- and α<sub>2</sub>-adrenoceptors are included in the contractile responses to lerimazoline. Lerimazoline-induced contractions were not affected by RX 821002, while they were inhibited by prazosin, with the change in the maximum response. To confirm that the α<sub>2</sub>-adrenoceptor is not involved in the contractile

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TABLE 4. Literature data on estimated binding affinities (K<sub>i</sub>) of eight antagonists used in the study. The values obtained for linear regression analysis are given in bold

<table>
<thead>
<tr>
<th>Receptor</th>
<th>PRAZOSIN</th>
<th>RX 821002</th>
<th>JP 1302</th>
<th>RAUWOLSCINE</th>
<th>SB 224289</th>
<th>BRL 15572</th>
<th>KETANSERIN</th>
<th>METHIOTHEPIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;-adrenoceptor</td>
<td>9.6</td>
<td>8.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α&lt;sub&gt;2&lt;/sub&gt;-adrenoceptor</td>
<td>-</td>
<td>9.2-9.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>α&lt;sub&gt;2C&lt;/sub&gt;-adrenoceptor</td>
<td>6.7-8.0</td>
<td>8.7</td>
<td>7.8</td>
<td>9.1</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>-</td>
<td>24.55</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.7*</td>
<td>-</td>
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<tr>
<td>Serotonin 5-HT&lt;sub&gt;1B&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.8</td>
<td>8.6*</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>6.27</td>
<td>7.9</td>
<td>7.4-7.5</td>
<td>7.3-8*</td>
<td>7.8-8.1</td>
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<td>-</td>
<td>-</td>
<td>5.3</td>
<td>6.6**</td>
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<td>7.8-8.4</td>
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<td>8.7-9.2</td>
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<td>6.8-7.5</td>
<td>8.4*</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>9.0-9.4</td>
<td>-</td>
</tr>
</tbody>
</table>

*Inverse agonist on specific type of receptor; **Full agonist on specific type of receptor; Data as compiled by The British Pharmacological Society (BPS) and the International Union of Basic and Clinical Pharmacology (IUPHAR). Available from: http://www.guidetopharmacology.org

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FIGURE 5. Effects of selective serotonergic antagonists: (A) 5-HT<sub>1D</sub> antagonist BRL 15572 (1 µM, n=8), (B) 5-HT<sub>1B</sub> antagonist SB 224289 (1 µM, n=10), and (C and D) 5HT<sub>2A</sub> antagonist ketanserin (1 µM, n=9; 10 nM, n=9) on the lerimazoline-induced contractions in the rat aorta. The points represent mean±standard error of the mean (SEM). The strength of constriction is expressed as a percentage of the maximum tension induced by lerimazoline to that induced by 10<sup>-4</sup> M phenylephrine. The effects of the antagonist on the lerimazoline-induced constrictions were compared with the control (*p<0.05, **p<0.01; Student’s t-test.)
response, the concentration-response curve of lerimazoline was also analyzed in the presence of rauwolscine, a non-selective α, adrenoceptor antagonist, and JP 1302, an α, adrenoceptor antagonist. Only rauwolscine produced displacement of the curve to the right, but without significantly changing the maximum contractile response to lerimazoline. Nevertheless, rauwolscine is also α, adrenoceptor antagonist in rats, as demonstrated in radioligand binding studies using rat cerebral cortex, with a low selectivity value for the α, adrenoceptor (5.5) [29]. Thus, our results with rauwolscine might be due to lerimazoline action at the α, rather than at α, adrenoceptor. Concordantly, intrahypothalamic injection of lerimazoline in rats, in doses up to 100 nmol, did not induce bradycardia, while clonidine, a selective α, adrenoceptor agonist, induced bradycardia at a dose of 7 nmol [17]. However, the selective α, adrenoceptor agonists phenylephrine and tramazoline also induced the bradycardic effects, though at higher doses (40 nmol for both); in addition, oxymetazoline, a putative non-selective α, adrenoceptor agonist, did not cause any effect at doses up to 100 nmol [17]. The described results could hardly be consistent if lerimazoline or oxymetazoline affects only the α-adrenoceptors.

Due to the observed inhibitory effect of methiothepin, a widely used non-selective 5-HT receptor antagonist, on the contractile response to lerimazoline, we performed additional experiments to investigate the possible involvement of the 5-HT2A, 5-HT2B, 5-HT2C, or 5-HT2D receptors in the lerimazoline-induced contractions. The blood vessels were pretreated with BRL 15572 or SB 224289, selective antagonists at the 5-HT2A and 5-HT2C receptor, respectively. Neither BRL 15572 nor SB 224289 reduced contraction to lerimazoline; the former, but not the latter finding is in agreement with the discussion above on the activity of lerimazoline on the rabbit saphenous vein [6]. As there are no data on the expression of 5-HT2A receptors in the rat aorta, it is possible that this receptor subtype was simply absent in the present model.

While both concentrations of ketanserin significantly reduced the contractile action of lerimazoline, we observed a significant positive correlation between literature Ki and our pKB values for the 5-HT2A receptor, when the values for 10 nM ketanserin were included, but not with the values obtained for 1 µM ketanserin. Previously, it was demonstrated that, at a lower concentration (10 nM), ketanserin antagonized only those receptors to which it has the highest affinity (pKi for the rat 5-HT2A was 8.6-9.0) [30], while at higher concentrations it additionally antagonized the α, adrenoceptor (pKi 7.8-8.2) [31]. Given the similarities with our results for ketanserin, a predominance of the 5-HT2A over α, adrenoergic receptors may be assumed.

In conclusion, our results suggest that the mechanism of vasoconstrictor action of lerimazoline encompasses both, the activation of 5-HT2A, and to a lesser degree α, adrenoergic receptors. These results also suggest that lerimazoline is an ‘atypical’ decongestant. Engaging multiple drug targets to achieve an optimal therapeutic outcome is a promising approach. Thus, the interactions of lerimazoline with phenylephrine as a standard decongestant should be further investigated, especially with respect to rebound congestion, which may be overcome by a polypharmacological approach to administration of decongestants with different mechanisms of action.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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Eldina Rizvić, et al.: Lerimazoline contracts rat aorta mainly through 5-HT2A receptors


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