

# Atypical sympathomimetic drug lerimazoline mediates contractile effects in rat aorta predominantly by 5-HT<sub>2A</sub> 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 D<sub>2</sub>, serotonin 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors and  $\alpha_1$ -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 ( $\alpha_1$ -adrenoceptor antagonist), RX 821002 and rauwolscine ( $\alpha_2$ -adrenoceptor antagonists), JP 1302 ( $\alpha_{2C}$ -adrenoceptor antagonist), methiothepin (non-selective 5-HT receptor antagonist), SB 224289 (5-HT<sub>1B</sub> receptor antagonist), BRL 15572 (5-HT<sub>1D</sub> receptor antagonist), and ketanserin (5-HT<sub>2A</sub> receptor antagonist). Lerimazoline displayed high affinity for the 5-HT<sub>1A</sub> receptor (K<sub>i</sub> = 162.5 nM), similar to the previously reported affinity for the 5-HT<sub>1D</sub> receptor. Binding affinity estimates (K<sub>i</sub>) for  $\alpha_1$ , 5-HT<sub>2A</sub>, and D<sub>2</sub> receptors were 6656, 4202 and 3437.5 nM, respectively (the literature reported K<sub>i</sub> for 5-HT<sub>1B</sub> 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  $\mu$ M) and methiothepin strongly suppressed the maximum response to lerimazoline. Overall, our results suggest that 5-HT<sub>2A</sub> and, less distinctly,  $\alpha_1$ -adrenergic receptors are involved in the lerimazoline-induced contractions, which makes lerimazoline an “atypical” decongestant.

KEY WORDS: Lerimazoline; rat aorta; binding affinity; phenylephrine; 5-HT<sub>2A</sub> receptors; St-71; trimizoline; tramazoline; antagonist activity; sympathomimetic drug

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## 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  $\alpha$ -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  $\alpha$ -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-HT<sub>2B</sub> 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 benefit of nasal decongestants. For example, the 5-HT<sub>1B</sub> receptors are expressed in human nasal mucosa and possibly play

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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 imidazoline class, has been used as a nasal decongestant for decades in several countries, in the concentration of  $2.1 \times 10^{-6}$  M for children and  $6.3 \times 10^{-6}$  M for adults [13]. However, the primary research studies on this compound, reported earlier as St-71, trimizoline or trimazoline, are scarce [14-17] and its pharmacological profile is not completely understood. While lerimazoline has a substantial affinity for the 5-HT<sub>1D</sub> and much lower affinity for 5-HT<sub>1B</sub> receptors (K<sub>i</sub> values 72 versus 3480 nM) [6], its affinity for other 5-HT or  $\alpha$ -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  $\alpha$ -adrenoceptor - and prostaglandin-independent manner [6].

Here, we analyzed the affinity of lerimazoline for the dopamine receptor D<sub>2</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors and  $\alpha_1$ -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  $10^{-10}$  M) of [3H] Spiperone [specific activity (spec. act.) 73.36 Ci mmol<sup>-1</sup>], 8-Hydroxy-[3H] DPAT (spec. act. 142 Ci mmol<sup>-1</sup>), [3H] Ketanserin (spec. act. 47.3 Ci mmol<sup>-1</sup>) and [3H] Prazosin (spec. act. 81.7 Ci mmol<sup>-1</sup>) were used to label the D<sub>2</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenoceptor, respectively, from rat striatal or cortical synaptosomes. Nonspecific binding was measured in the presence of 10  $\mu$ M of spiperone (D<sub>2</sub>), 10  $\mu$ M of buspirone (5-HT<sub>1A</sub>), 10  $\mu$ M of ketanserin (5-HT<sub>2A</sub>), and 10  $\mu$ M of prazosin ( $\alpha_1$ ). The tubes (0.4 ml total volume of the incubation mixture for D<sub>2</sub> receptor and 0.2 ml total volume of the incubation mixture for 5HT<sub>1A</sub>, 5-HT<sub>2A</sub>, and  $\alpha_1$ -adrenergic receptor) 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<sub>1B</sub> receptor antagonist), BRL 15572 (5-HT<sub>1D</sub> receptor antagonist) and RX 821002 hydrochloride ( $\alpha_2$ -adrenoceptor antagonist) were purchased from Tocris (Bristol, UK). Ketanserin tartrate (5-HT<sub>2A</sub> receptor antagonist), methiothepin hydrochloride (non-selective 5-HT receptor antagonist), prazosin hydrochloride ( $\alpha_1$ -adrenoceptor antagonist), rauwolscine hydrochloride ( $\alpha_2$ -adrenoceptor antagonist) and JP 1302 hydrochloride ( $\alpha_{2C}$ -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  $22 \pm 1^\circ\text{C}$ , 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<sub>2</sub>) 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 CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub> × 7H<sub>2</sub>O, 25 mM NaHCO<sub>3</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub> and 11 mM glucose, maintained at 37°C, pH 7.4, continuously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

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/4SP 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<sup>-4</sup> M) followed by the addition of acetylcholine (10<sup>-5</sup> 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<sup>-7-3</sup> × 10<sup>-4</sup> 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<sup>-7-3</sup> × 10<sup>-4</sup> 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 224289 (10<sup>-6</sup> M), BRL 15572 (10<sup>-6</sup> M), ketanserin tartrate (10<sup>-8</sup> and 10<sup>-6</sup> M), prazosin hydrochloride (3 × 10<sup>-7</sup> M), RX 821002 (3 × 10<sup>-9</sup> M), rauwolfscine hydrochloride (10<sup>-7</sup> M) or JP 1302 (10<sup>-5</sup> 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<sup>-4</sup> M).

The strength of constriction was expressed as a percentage of the maximum tension induced by lerimazoline compared to that induced by phenylephrine (10<sup>-4</sup> 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<sup>-4</sup> M). The potency of the agonist was measured in terms of EC<sub>50</sub> for each curve (the given concentration of lerimazoline was required to produce 50% of the calculated maximum response for the agonist) and presented as pEC<sub>50</sub> (the negative log<sub>10</sub> of the EC<sub>50</sub> value) by using software LabChart 7 (ADInstruments). Antagonist potency was expressed as an apparent pK<sub>b</sub> value calculated according to the following equation:

$$pK_b = \log(DR-1) - \log[B],$$

where DR is the ratio of the mean EC<sub>50</sub> value in the presence of the antagonist to the mean EC<sub>50</sub> 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 serotonergic receptors in humans (or if available in the rat) and our pK<sub>b</sub> 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 (K<sub>i</sub>) of lerimazoline for four examined receptor types (D<sub>2</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, and α<sub>1</sub>-adrenoceptor) are presented in Table 1. Among these receptors, lerimazoline displayed the highest submicromolar affinity for the 5-HT<sub>1A</sub> 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<sub>1D</sub> and 5-HT<sub>1B</sub> receptors [6] were also reproduced (designated with an asterisk)

Receptor	Ki±SEM (nM)
Dopamine D <sub>2</sub>	3437.5±138.9
Serotonin 5-HT <sub>1A</sub>	162.5±4.5
Serotonin 5-HT <sub>2A</sub>	4202±78
α <sub>1</sub> -adrenoceptor	6656±149
Serotonin 5-HT <sub>1D</sub> *	72±12
Serotonin 5-HT <sub>1B</sub> *	3480±1690

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

preparations, in the concentration range  $3 \times 10^{-6}$ - $3 \times 10^{-4}$  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 ( $E_{max}$ :  $45.08 \pm 1.18\%$  of  $10^{-4}$  phenylephrine maximal contraction;  $pEC_{50}$ : 4.30,  $n = 75$ ; the  $pEC_{50}$  of 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  $3 \times 10^{-4}$  M. However, a number of experiments using additional concentrations ( $1 \times 10^{-3}$  and  $3 \times 10^{-3}$  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  $3 \times 10^{-4}$  M.

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

To examine the involvement of noradrenergic (α<sub>1</sub>- and α<sub>2</sub>-adrenoceptor) and serotonergic systems in lerimazoline-induced contraction, selective α-adrenoceptor and serotonergic antagonists were used in the rat aortas (Table 3).

Prazosin (0.3 μM), an α<sub>1</sub>-adrenoceptor antagonist, significantly reduced the maximum contractile response to lerimazoline ( $E_{max}$ :  $44.83 \pm 3.28\%$  in the absence versus  $27.83 \pm 5.32\%$  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 pK<sub>b</sub> value because DR was less than 1.

RX 821002 (30 nM, pK<sub>b</sub> = 8.19), an α<sub>2</sub>-adrenoceptor antagonist, did not affect significantly the contractile response to

**TABLE 2.** Effects of lerimazoline on the constriction of rat thoracic aorta

Lerimazoline (M)	Constriction (%)
$1 \times 10^{-7}$	-3.07±0.38
$3 \times 10^{-7}$	-1.70±0.49
$1 \times 10^{-6}$	-0.86±0.56
$3 \times 10^{-6}$	1.68±0.73
$1 \times 10^{-5}$	5.69±0.93
$3 \times 10^{-5}$	16.42±1.25
$1 \times 10^{-4}$	29.80±1.34
$3 \times 10^{-4}$	45.08±1.18

The strength of constriction is expressed as a percentage of the maximum tension induced by lerimazoline compared to that induced by  $10^{-4}$  M phenylephrine. Data are expressed as mean±standard error of the mean (SEM),  $n=75$

**TABLE 3.** Effect of prazosin, RX 821002, rauwolscine, JP 1302, ketanserin, methiothepin, SB 224289, and BRL 15572 on the contractions of rat thoracic aorta induced by lerimazoline

Drug	pEC <sub>50</sub>	n	Concentration (μM)
PRAZOSIN			
Untreated	3.93±0.09	15	0.3
Treated	4.01±0.24		
RX 821002			
Untreated	3.91±0.26	12	0.03
Treated	3.15±0.27		
PRAZOSIN+RX 821002			
Untreated	3.45±0.16	8	0.3
Treated	3.51±0.37		0.03
JP 1302			
Untreated	4.07±0.11	9	1
Treated	3.98±0.35		
RAUWOLSCINE			
Untreated	4.13±0.13	10	0.1
Treated	3.69±0.11		
METHIOTHEPIN			
Untreated	4.30±0.13	9	0.3
Treated	3.99±0.34		
BRL 15572			
Untreated	3.85±0.14	12	1
Treated	3.77±0.21		
SB 224289			
Untreated	4.24±0.08	10	1
Treated	3.98±0.08		
KETANSERIN			
Untreated	4.42±0.24	9	1
Treated	3.80±0.27		
KETANSERIN			
Untreated	4.02±0.31	7	0.01
Treated	3.89±0.38		

Values are expressed as mean±standard error of the mean (SEM); pEC<sub>50</sub> is the negative log of lerimazoline concentrations inducing 50% of the maximum contractile response

lerimazoline ( $E_{max}$ :  $40.92 \pm 3.65\%$  in the absence versus  $35.45 \pm 6.31\%$  in the presence of RX 821002,  $p > 0.05$ , Figure 2B;  $n = 12$ ).

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 ( $E_{max}$ :  $48.72 \pm 4.86\%$  in the absence versus  $28.81 \pm 6.80\%$  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 pK<sub>b</sub> value (pEC<sub>50</sub> value for lerimazoline was  $3.45 \pm 0.16$ , while for

the combination of prazosin and RX 821002 it was  $3.51 \pm 0.37$ ;  $p > 0.05$ ).

Next, we examined the effect of the nonselective  $\alpha_2$ -adrenoceptor antagonist rauwolscine and  $\alpha_{2C}$ -adrenoceptor antagonist JP 1302 on the isolated rat aorta. While JP 1302 ( $1 \mu\text{M}$ ;  $pK_b = 5.4$ ) did not significantly affect the lerimazoline-induced contraction ( $E_{\text{max}}$ :  $37.85 \pm 4.03\%$  in the absence versus  $36.39 \pm 5.23\%$

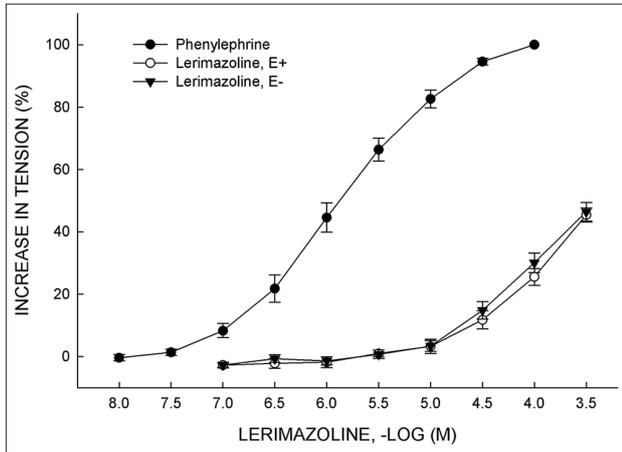
in the presence of JP 1302; Figure 3B;  $n = 9$ ), rauwolscine ( $0.1 \mu\text{M}$ ;  $pK_b = 7.23$ ) caused a rightward shift in the concentration-response curve to lerimazoline, but the result was not significant ( $38.82 \pm 3.98$  in the absence versus  $27.66 \pm 4.14\%$  in the presence of rauwolscine,  $p = 0.067$ , Figure 3A;  $n = 10$ ).

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

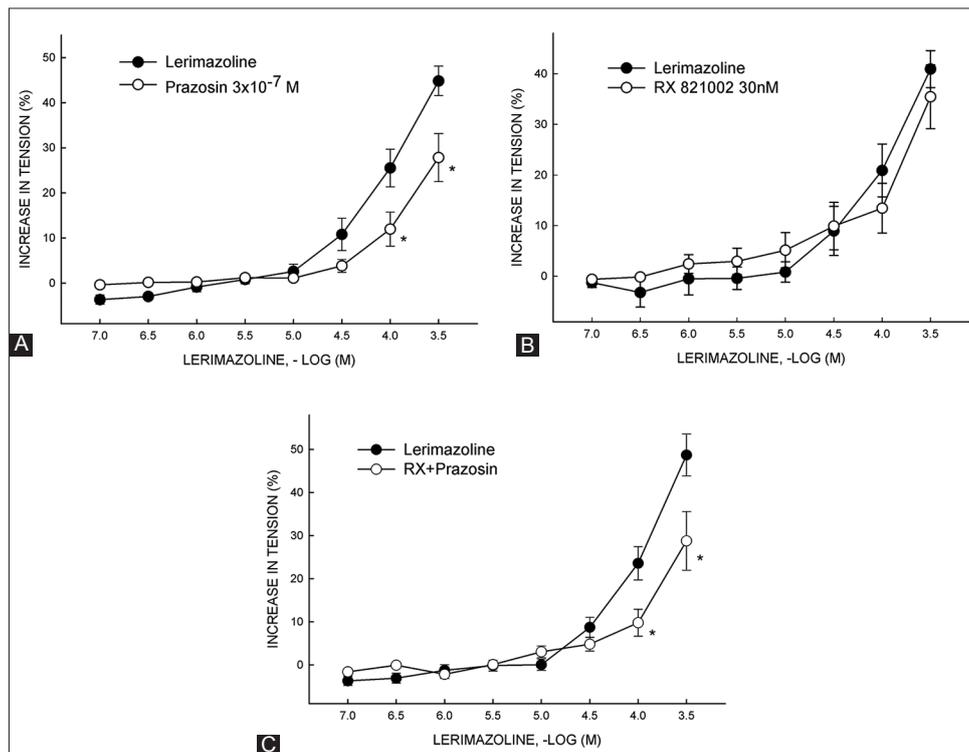
Methiothepin (a non-selective serotonergic antagonist,  $30 \text{ nM}$ ;  $pK_b = 7.53$ ) strongly suppressed the maximum response to lerimazoline [ $E_{\text{max}}$ :  $46.26 \pm 6.06\%$  in the absence versus  $14.58 \pm 5.57\%$  in the presence of methiothepin,  $p < 0.01$ ] (Figure 4).

The effects of the selective 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, and 5-HT<sub>2A</sub> antagonists are shown in Figure 5. 5-HT<sub>1D</sub> antagonist BRL 15572 did not block the response to lerimazoline at  $1 \mu\text{M}$  ( $pK_b = 5.28$ ;  $E_{\text{max}}$ :  $43.63 \pm 3.58\%$  in the absence versus  $33.73 \pm 7.02\%$  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<sub>1B</sub> antagonist SB 224289 at  $1 \mu\text{M}$  ( $pK_b = 5.92$ ;  $E_{\text{max}}$ :  $38.04 \pm 3.61\%$  in the absence versus  $33.61 \pm 6.12\%$  in the presence of SB 224289,  $p > 0.05$ , Figure 5B;  $n = 10$ ).

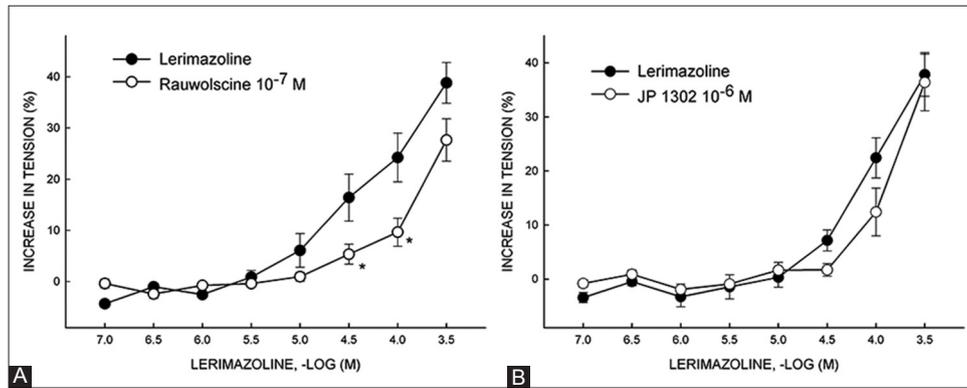
Since the rat thoracic aorta is an established model for 5-HT<sub>2A</sub> receptor-mediated contraction [26], we examined the effect of ketanserin at two concentrations:  $10 \text{ nM}$  ( $pK_b = 7.53$ ) and  $1 \mu\text{M}$  ( $pK_b = 6.51$ ). At  $1 \mu\text{M}$  concentration, ketanserin



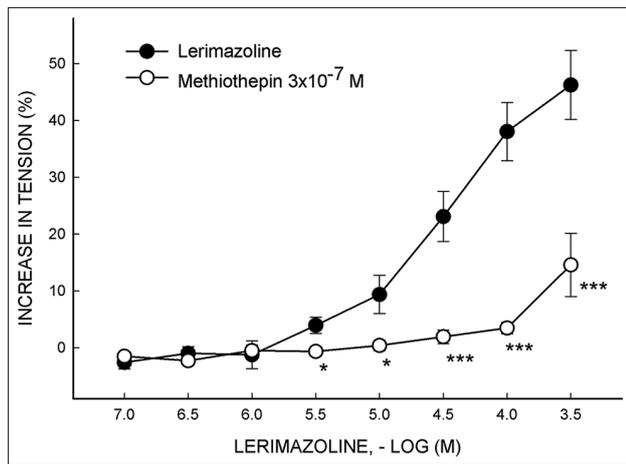
**FIGURE 1.** Concentration-response curves for phenylephrine ( $n=12$ ) and lerimazoline ( $n=38$ ) in the rat aorta with and without endothelium. Values are expressed as mean  $\pm$  standard error of the mean (SEM). The strength of constriction is expressed as a percentage of the maximum tension induced by lerimazoline to that induced by  $10^{-4} \text{ M}$  phenylephrine. The removal of the endothelium did not affect the contraction induced by lerimazoline.



**FIGURE 2.** Effects of (A)  $\alpha_1$ -adrenoceptor antagonist prazosin ( $0.3 \mu\text{M}$ ,  $n=15$ ), (B)  $\alpha_2$ -adrenoceptor antagonist RX 821002 ( $30 \text{ nM}$ ,  $n=12$ ) and (C) the combination of prazosin and RX 821002 ( $n=9$ ) on lerimazoline-induced contraction in the rat aorta. The points represent mean  $\pm$  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  $10^{-4} \text{ M}$  phenylephrine. The effects of the antagonist(s) on the lerimazoline-induced constrictions were compared with the control ( $*p < 0.05$ , Student's  $t$ -test).



**FIGURE 3.** Effects of (A) non-selective  $\alpha_2$ -adrenoceptor antagonist rauwolscine (0.1  $\mu\text{M}$ ,  $n=10$ ) and (B)  $\alpha_2\text{C}$ -adrenoceptor antagonist JP 1302 (1  $\mu\text{M}$ ,  $n=9$ ) on the lerimazoline-induced contractions in the rat aorta. The points represent mean  $\pm$  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  $10^{-4}$  M phenylephrine. The effects of the antagonist on the lerimazoline-induced constrictions were compared with the control ( $*p < 0.05$ , Student's  $t$ -test).



**FIGURE 4.** Effects of non-selective 5-HT-receptor antagonist methiothepin at the concentration of 0.3  $\mu\text{M}$  ( $n=9$ ) on the lerimazoline-induced contractions in the rat aorta. The points represent mean  $\pm$  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  $10^{-4}$  M phenylephrine. The effects of the antagonist on the lerimazoline-induced constrictions were compared with the control ( $*p < 0.05$ ,  $***p < 0.001$ , Student's  $t$ -test).

significantly inhibited lerimazoline-evoked vasoconstriction [lerimazoline concentrations  $> 10 \mu\text{M}$ ] (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  $\mu\text{M}$  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 $K_i$ values from the literature

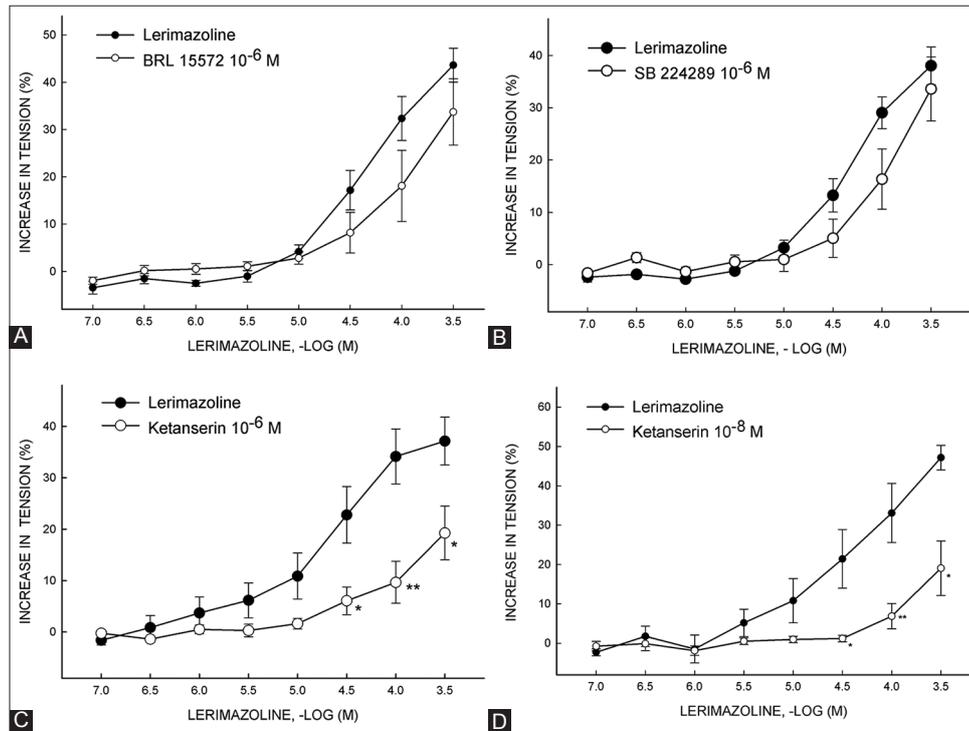
Three or more pairs of valid data, necessary to determine linear correlation between  $K_i$  (literature data given in Table 4) and calculated  $\text{pKb}$  values were found for the four receptor subtypes; the analysis for 5-HT<sub>1D</sub> and 5-HT<sub>2A</sub> receptors was

performed twice, involving separately the  $\text{pKb}$  values calculated for ketanserin at 10 nM and 1  $\mu\text{M}$  concentration, respectively. A total of 6 correlations were obtained, but only one was statistically significant. For the 5-HT<sub>2A</sub> receptor a high and significant correlation with the literature values was observed only with the  $\text{pKb}$  value for 10 nM ketanserin ( $r = 0.997$ ,  $n = 3$ ,  $p = 0.049$ ), but not with 1  $\mu\text{M}$  of ketanserin ( $r = 0.729$ ,  $n = 3$ ,  $p = 0.480$ ). A significant correlation was not observed for the  $\alpha_2\text{C}$ -adrenoceptor ( $r = 0.796$ ,  $n = 3$ ,  $p = 0.414$ ), 5-HT<sub>1A</sub> receptor ( $r = 0.906$ ,  $n = 3$ ,  $p = 0.278$ ), and 5-HT<sub>1D</sub> receptor; when both  $\text{pKb}$  values of ketanserin were included (for 1  $\mu\text{M}$  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<sub>1D</sub> receptors ( $K_i = 72$  nM) and with micromolar affinity to the 5-HT<sub>1B</sub> receptors ( $K_i = 3480$  nM) [6]. In cells expressing recombinant 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> 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  $\alpha_1$ -,  $\alpha_2$ -adrenergic or prostaglandin-mediated effects, respectively. The  $\text{EC}_{50}$  of lerimazoline (150 nM) was similar to that of the 5-HT<sub>1B/D</sub> receptor agonist sumatriptan (220 nM) [6]. In another study, treatment with sumatriptan, a 5-HT<sub>1D</sub> and 5-HT<sub>1B</sub> receptor antagonist, in rabbit saphenous vein implied that contractile action of lerimazoline on this vein is predominantly associated with the activation of 5-HT<sub>1B</sub> receptors [27].



**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).

**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

Receptor	PRAZOSIN	RX 821002	JP 1302	RAUWOLSCINE	SB 224289	BRL 15572	KETANSERIN	METHIOHEPIN
α <sub>1</sub> -adrenoceptor	9.6	8.2	-	-	-	-	8.2	-
α <sub>2</sub> -adrenoceptor	-	9.2-9.5	-	8.4	-	-	-	-
α <sub>2C</sub> -adrenoceptor	6.7-8.0	8.7	7.8	9.1	-	-	-	-
Serotonin 5-HT <sub>1A</sub>	-	24.55	-	-	-	7.7*	-	7.8-8.1
Serotonin 5-HT <sub>1B</sub>	-	-	-	-	8.6*	-	-	7.1-8.5*
Serotonin 5-HT <sub>1D</sub>	-	-	-	7.8	6.27	7.9	7.4-7.5	7.3-8.2*
Serotonin 5-HT <sub>2A</sub>	-	-	-	-	5.3	6.6**	8.6-9.0	8.5
Serotonin 5-HT <sub>2B</sub>	-	-	-	7.8-8.4	-	-	-	8.7-9.2
Serotonin 5-HT <sub>2C</sub>	-	-	-	-	-	-	6.8-7.5	8.4*
Serotonin 5-HT <sub>7</sub>	-	-	-	-	-	-	-	9.0-9.4

\*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>

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 receptor subtypes suggests that the 5-HT<sub>1A</sub> and 5-HT<sub>1D</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

response, the concentration-response curve of lerimazoline was also analyzed in the presence of rauwolscine, a non-selective  $\alpha_2$ -adrenoceptor antagonist, and JP 1302, an  $\alpha_{2C}$ -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  $\alpha_1$ -adrenoceptor antagonist in rats, as demonstrated in radioligand binding studies using rat cerebral cortex, with a low selectivity value for the  $\alpha_2$ -adrenoceptor (5.5) [29]. Thus, our results with rauwolscine might be due to lerimazoline action at the  $\alpha_1$ - rather than at  $\alpha_2$ -adrenoceptor. Concordantly, intrahypothalamic injection of lerimazoline in rats, in doses up to 100 nmol, did not induce bradycardia, while clonidine, a selective  $\alpha_2$ -adrenoceptor agonist, induced bradycardia at a dose of 7 nmol [17]. However, the selective  $\alpha_1$ -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  $\alpha$ -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  $\alpha$ -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-HT<sub>1A</sub>, 5-HT<sub>1B/1D</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> or 5-HT<sub>2C</sub> receptors in the lerimazoline-induced contractions. The blood vessels were pretreated with BRL 15572 or SB 224289, selective antagonists at the 5-HT<sub>1D</sub> and 5-HT<sub>1B</sub> 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-HT<sub>1B</sub> receptors in the rat aorta, it is possible that this receptor substrate 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  $K_i$  and our pK<sub>b</sub> values for the 5-HT<sub>2A</sub> receptor, when the values for 10 nM ketanserin were included, but not with the values obtained for 1  $\mu$ M ketanserin. Previously, it was demonstrated that, at a lower concentration (10 nM), ketanserin antagonized only those receptors to which it has the highest affinity (pK<sub>i</sub> for the rat 5-HT<sub>2A</sub> was 8.6-9.0) [30], while at higher concentrations it additionally antagonized the  $\alpha_1$ -adrenoceptor (pK<sub>i</sub> 7.8-8.2) [31]. Given the similarities with our results for ketanserin, a predominance of the 5-HT<sub>2A</sub> over  $\alpha_1$ -adrenergic receptors may be assumed.

In conclusion, our results suggest that the mechanism of vasoconstrictor action of lerimazoline encompasses both,

the activation of 5-HT<sub>2A</sub>, and to a lesser degree  $\alpha_1$ -adrenergic 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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