Cardiovascular Effects of Clonidine-Like Drugs in Pithed Rabbits

Kenia Pompermayer, Maria Cristina O. Salgado, Josiane Feldman, Pascal Bousquet

Abstract—Administration (3 to 100 μg/kg IV) of clonidine, rilmenidine, and an imidazoline derivative, 2-(2-chlorophenylamino)imidazoline, in pithed nonstimulated rabbits caused a dose-dependent increase in mean arterial pressure without affecting heart rate. Prazosin (0.1 mg/kg IV) almost abolished the pressor responses to 2-(2-chlorophenylamino)imidazoline, partially inhibited those induced by clonidine, but failed to affect those elicited by rilmenidine. In contrast, yohimbine (1 mg/kg IV) blunted the pressor responses of the 3 drugs. In sympathetically stimulated pithed rabbits, 2-(2-chlorophenylamino)imidazoline induced only pressor effects, whereas clonidine and rilmenidine caused a transient pressure increase followed by a dose-dependent depressor effect. Yohimbine abolished the depressor effect of both drugs, which may have involved presynaptic α₂-adrenoceptors. In conclusion, peripheral effects of 2-(2-chlorophenylamino)imidazoline and clonidine involved at least α₁- and α₂-adrenoceptor activation, whereas pressor and depressor effects of rilmenidine were mediated by α₂-adrenoceptors. (Hypertension. 1999;34[part 2]:1012-1015.)

Key Words: adrenergic receptors ■ sympathetic ■ antihypertensive agents

Clonidine and rilmenidine are centrally acting antihypertensive drugs that contain an imidazoline or an oxazoline ring, respectively, in their chemical structures. The site of the hypotensive action of clonidine was first localized in the nucleus reticularis lateralis of the rostroventrolateral medulla.¹ A structure-activity relationship study showed that imidazolines were able to induce hypotension when applied to this region, whereas catecholamines do not change blood pressure. These results suggest the existence of imidazolinesensitive receptors involved in the hypotensive effect of imidazoline drugs and related substances.²,³

Although the hypotensive effect of clonidine-like drugs involves central imidazoline receptors, many studies suggest the possibility of a peripheral presynaptic contribution to the depression of sympathetic tone produced by these drugs.⁴,⁵ In this regard, presynaptic α₂-adrenoceptors have been shown to be involved in the inhibition of norepinephrine release.⁶,⁷ It was recently suggested that inhibitory presynaptic imidazoline receptors may also be involved in this response.⁸,⁹

The aim of the present work was to investigate and to compare the presynaptic and postsynaptic effects of clonidine, rilmenidine, and an imidazoline derivative, 2-(2-chlorophenylamino)imidazoline, in the peripheral cardiovascular system of pithed rabbits with or without electrically stimulated sympathetic outflow.

Methods

The experiments were performed on pithed male rabbits (2.5 to 3 kg) with or without electrically stimulated sympathetic outflow as previously described by Szabo et al.¹⁰ All experiments were conducted in accordance with institutional guidelines on the use of animals in research. The animals were anesthetized with sodium pentobarbital (70 mg/kg IV), and the trachea was cannulated to allow artificial respiration. Catheters were placed into the right jugular vein and carotid artery for drug administration and arterial pressure measurement, respectively. Heart rate was calculated from the pressure signal recorded by a pressure transducer (HP-1280C) and connected to a computer measuring program (DATAQ-DI220-Windaq). Pancuronium bromide (2 mg/kg) was given intravenously to prevent skeletal muscle contraction. The rabbits were pithed by insertion of a steel rod (3.5 mm in diameter) through a hole in the parietal bone 25 cm down the spinal canal. The rod destroyed the central nervous system and also served to electrically stimulate the peripheral sympathetic nerves with a stimulator (20 V, 2 Hz, 0.5 ms). An indifferent electrode was placed subcutaneously between the scapulae.

In all experiments, a 60-minute period was allowed before any drug injection. Vehicle administration produced no significant effect on hemodynamic parameters. Increasing doses of drugs were injected at 15-minute intervals. When antagonists were used, prazosin (0.1 mg/kg), yohimbine (1 mg/kg), or a combination of the two was injected 10 minutes before the injection of the agonists.

Drugs used were clonidine (RBI), rilmenidine (Servier), 2-(2-chlorophenylamino)imidazoline (synthesized in the Strasbourg laboratory), prazosin hydrochloride (Sigma), yohimbine hydrochloride (Sigma), sodium pentobarbital (Nembutal, Sigma), and pancuronium bromide (Cristalia). Drugs were dissolved in normal saline.

Results were expressed as mean±SEM. Differences between groups were determined by 2-factor ANOVA with repeated measures followed by Student’s t test with Bonferroni corrections. Differences within each group were determined by ANOVA and repeated measures followed by Student’s t test. A value of P<0.05 was considered statistically significant.

Received May 8, 1999; first decision June 15, 1999; revision accepted July 13, 1999. From the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil, and the Laboratoire de Neurobiologie et Pharmacologie Cardiovasculaire, Faculté de Médecine, ULP, Strasbourg, France. Correspondence to Maria Cristina O. Salgado, PhD, Department of Pharmacology, School of Medicine-USP, 14049-900 Ribeirão Preto, SP, Brazil. E-mail mcdosalg@fmrp.usp.br

© 1999 American Heart Association, Inc. Hypertension is available at http://www.hypertensionaha.org
Results

Pithed Rabbits Without Electrical Stimulation

Basal mean arterial pressure and heart rate were 33±1 mm Hg and 205±4 bpm (n=47), respectively. Injections (3 to 100 µg/kg IV) of clonidine, rilmenidine, or 2-(2-chlorophenylamino)imidazoline induced dose-dependent increases in mean arterial pressure with different maximal pressor activity (Figure 1). At the highest dose, 100 µg/kg, rilmenidine induced the lowest pressor effect (84±10% increase in pressure, n=11), followed by 2-(2-chlorophenylamino)imidazoline (160±1%, n=10) and clonidine (221±19%, n=11). In these conditions, heart rate was not affected by these drugs. Basal arterial pressure and heart rate were not significantly affected by administration of prazosin or yohimbine. Pressor responses to clonidine (Figure 1A) were partially reduced by yohimbine, whereas prazosin reduced these responses only when high doses were used. Pressor responses induced by rilmenidine (Figure 1B) were unaffected by prazosin but were significantly reduced by yohimbine or a combination of the two antagonists. In contrast, pressor responses to 2-(2-chlorophenylamino)imidazoline (Figure 1C) were almost abolished by prazosin alone, whereas yohimbine only partially reduced these responses.

Pithed Rabbits With Electrical Stimulation

Peripheral effects of clonidine, rilmenidine, and 2-(2-chlorophenylamino)imidazoline were also studied in pithed rabbits with preganglionic electrical stimulation of sympathetic nerves. Stimulation induced a significant increase in basal mean arterial pressure (63±3 mm Hg) and heart rate (266±8 bpm) compared with the nonstimulated group (P<0.05, n=26). Clonidine (15 to 1000 µg/kg) and rilmenidine (15 to 1000 µg/kg) caused a transient increase in mean arterial pressure, followed by a dose-dependent depressor effect (Figure 2A and 2B) and reduction in heart rate (Table). In contrast, 2-(2-chlorophenylamino)imidazoline (15 to 1000 µg/kg) induced only a dose-dependent pressor effect (Figure 2C), without significant changes in heart rate (Table).

To determine the contribution of α₁-adrenoceptors to the biphasic effect of clonidine and rilmenidine, yohimbine (1 mg/kg IV) was administered 10 minutes before the tested drugs. Yohimbine did not change heart rate but induced a

Figure 1. Effects of yohimbine (1 mg/kg IV), prazosin (0.1 mg/kg IV), or yohimbine plus prazosin on mean arterial pressure increase induced by clonidine (A), rilmenidine (B), and 2-(2-chlorophenylamino)imidazoline (C) in pithed rabbits. Data are mean±SEM. *P<0.05 vs control.

Figure 2. Effects of intravenously administered clonidine (n=5), rilmenidine (n=5), and 2-(2-chlorophenylamino)imidazoline (n=4) on mean arterial pressure of pithed rabbits with electrically stimulated sympathetic outflow. Data are mean±SEM.
Effects of Clonidine (C), Rilmenidine (R), and 2-(2-Chlorophenylamino)imidazoline (I) on Heart Rate in Pithed Rabbits Submitted to Electrical Stimulation of Sympathetic Nerves in Control Conditions and After Yohimbine

<table>
<thead>
<tr>
<th>Dose, μg/kg</th>
<th>Control</th>
<th>Yohimbine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>R</td>
</tr>
<tr>
<td>Basal</td>
<td>254±16</td>
<td>250±21</td>
</tr>
<tr>
<td>15</td>
<td>236±13</td>
<td>236±10</td>
</tr>
<tr>
<td>30</td>
<td>232±13</td>
<td>228±14</td>
</tr>
<tr>
<td>100</td>
<td>228±10*</td>
<td>210±13*</td>
</tr>
<tr>
<td>300</td>
<td>218±10*</td>
<td>212±14*</td>
</tr>
<tr>
<td>1000</td>
<td>210±9*</td>
<td>206±15*</td>
</tr>
</tbody>
</table>

Values are bpm, mean±SEM (n=5 or 6).

*P<0.05 vs control.

In the present study, we compared the cardiovascular effects of clonidine, rilmenidine, and the imidazoline derivative 2-(2-chlorophenylamino)imidazoline given systemically to pithed rabbits with or without electrically stimulated sympathetic outflow.

The potent vasopressor effects of clonidine and 2-(2-chlorophenylamino)imidazoline in nonstimulated rabbits were significantly inhibited by yohimbine and prazosin, indicating that these compounds produced vasoconstriction by stimulation of postsynaptic vascular α₁- and α₂-adrenoceptors. The pressor effects of low doses of clonidine were mainly due to stimulation of α₂-adrenoceptors, whereas additional stimulation of α₁-adrenoceptors occurred at higher doses. This observation confirmed previous observations in pithed rats. The weak pressor effects of rilmenidine were significantly inhibited by yohimbine and were unaffected by prazosin, suggesting that rilmenidine exhibited some selectivity for postsynaptic vascular α₁-adrenoceptors.

The presynaptic α₂-adrenergic activity of clonidine and rilmenidine was confirmed in stimulated pithed rabbits; in these conditions, these compounds produced a hypertensive effect, followed by depressor and bradycardic effects. Yohimbine prevented the depressor effects of both drugs, suggesting that these effects were probably mediated by presynaptic α₂-adrenoceptor. The pressor effects of clonidine were not affected by yohimbine, whereas those induced by rilmenidine were significantly reduced. These results confirmed the selectivity for α₂-adrenoceptors of rilmenidine observed in the nonstimulated animals. The fact that the maximal depressor effect of rilmenidine, observed in stimulated animals, was much weaker than that of clonidine strongly suggests that rilmenidine acted on presynaptic α₂-adrenoceptors as a partial agonist. Such an effect might also explain that rilmenidine caused α₂-adrenoceptor–mediated vasoconstriction that was also weaker than that of clonidine. Surprisingly, bradycardia induced by clonidine was insensitive to yohimbine. In contrast, studies performed in pithed rats have shown that clonidine inhibited electrically induced tachycardia and that this effect was sensitive to α₂-adrenoceptor antagonists. Recently, the presence of a presynaptic imidazoline receptor has been suggested in the human heart, as well as in rabbit aorta and pulmonary arteries and rat vena cava. Our results do not exclude the possibility that rilmenidine and clonidine elicit effects through a putative presynaptic imidazoline receptor in sympathetic stimulated pithed rabbits. Experiments with selective

Discussion

In the present study, we compared the cardiovascular effects of clonidine, rilmenidine, and the imidazoline derivative 2-(2-chlorophenylamino)imidazoline given systemically to pithed rabbits with or without electrically stimulated sympathetic outflow.

The potent vasopressor effects of clonidine and 2-(2-chlorophenylamino)imidazoline in nonstimulated rabbits were significantly inhibited by yohimbine and prazosin, indicating that these compounds produced vasoconstriction by stimulation of postsynaptic vascular α₁- and α₂-adrenoceptors. The pressor effects of low doses of clonidine were mainly due to stimulation of α₂-adrenoceptors, whereas additional stimulation of α₁-adrenoceptors occurred at higher doses. This observation confirmed previous observations in pithed rats. The weak pressor effects of rilmenidine were significantly inhibited by yohimbine and were unaffected by prazosin, suggesting that rilmenidine exhibited some selectivity for postsynaptic vascular α₁-adrenoceptors.

The presynaptic α₂-adrenergic activity of clonidine and rilmenidine was confirmed in stimulated pithed rabbits; in these conditions, these compounds produced a hypertensive effect, followed by depressor and bradycardic effects. Yohimbine prevented the depressor effects of both drugs, suggesting that these effects were probably mediated by presynaptic α₂-adrenoceptor. The pressor effects of clonidine were not affected by yohimbine, whereas those induced by rilmenidine were significantly reduced. These results confirmed the selectivity for α₂-adrenoceptors of rilmenidine observed in the nonstimulated animals. The fact that the maximal depressor effect of rilmenidine, observed in stimulated animals, was much weaker than that of clonidine strongly suggests that rilmenidine acted on presynaptic α₂-adrenoceptors as a partial agonist. Such an effect might also explain that rilmenidine caused α₂-adrenoceptor–mediated vasoconstriction that was also weaker than that of clonidine. Surprisingly, bradycardia induced by clonidine was insensitive to yohimbine. In contrast, studies performed in pithed rats have shown that clonidine inhibited electrically induced tachycardia and that this effect was sensitive to α₂-adrenoceptor antagonists. Recently, the presence of a presynaptic imidazoline receptor has been suggested in the human heart, as well as in rabbit aorta and pulmonary arteries and rat vena cava. Our results do not exclude the possibility that rilmenidine and clonidine elicit effects through a putative presynaptic imidazoline receptor in sympathetic stimulated pithed rabbits. Experiments with selective
antagonists are needed to further clarify our observations in pithed rabbits.

In nonstimulated rabbits, the vasoconstrictive effect of 2-(2-chlorophenylamino)imidazoline was more sensitive to prazosin than to yohimbine. Consistent with this observation is the fact that this derivative, like phenylephrine (data not shown), produced neither depressor nor bradycardic effects in stimulated pithed rabbits. Our results show that a very weak chemical change, the absence of a chloride atom in position 6 on the aromatic ring, largely altered the pharmacological profile of the drug.

In conclusion, peripheral effects of clonidine and 2-(2-chlorophenylamino)imidazoline involve \( \alpha_1 \) and \( \alpha_2 \)-adrenoceptor activation. The weak pressor and depressor effects of rilmenidine in pithed rabbits with or without stimulation suggest that this drug acts as a partial agonist on peripheral \( \alpha_2 \)-adrenoceptors.

Acknowledgments

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), and K.P. is the recipient of a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes). The authors thank Osmar Vettore for expert technical assistance.

References


8. Molderings GJ, Göthert M. Inhibitory presynaptic imidazoline receptors on sympathetic nerves in the rabbit aorta differ from \( \alpha_1 \)- and \( \alpha_2 \)-imidazoline binding sites. *Naunyn Schmiedebergs Arch Pharmacol*. 1995;351:507–516.


Cardiovascular Effects of Clonidine-Like Drugs in Pithed Rabbits
Kenia Pompermayer, Maria Cristina O. Salgado, Josiane Feldman and Pascal Bousquet

Hypertension. 1999;34:1012-1015
doi: 10.1161/01.HYP.34.4.1012

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/34/4/1012

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/