The Hypotensive Action of Rilmenidine is Dependent on Functional N-Methyl-d-aspartate Receptor in the Rostral Ventrolateral Medulla of Conscious Spontaneously Hypertensive Rats

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ABSTRACT

Rilmenidine, a selective imidazoline (I1) receptor agonist (Ernsberger et al., 1992; Reis, 1996), exhibits fewer side effects compared with α2-adrenergic receptor agonists such as clonidine (van Zwieten et al., 1986; Haxhiu et al., 1994). The major site of rilmenidine action is the rostroventrolateral medulla oblongata (RVLM) (Punnen et al., 1987; Ernsberger et al., 1988; Gomez et al., 1991; Haxhiu et al., 1994; Reis, 1996), an important area for cardiovascular regulation (Dampney et al., 1982; Kubo et al., 1993). However, the mechanism of rilmenidine action on blood pressure is not fully understood.

The excitatory amino acid L-glutamate plays an important role in cardiovascular regulation by activating two families of glutamate receptors, the metabotropic and the ionotropic glutamate receptors. The N-methyl-d-aspartate receptor (NMDAR) belongs to the latter family (Pin and Duvoisin, 1995). In the RVLM, the NMDAR mediates the pressor response induced by carotid body chemoreceptor (Kubo et al., 1993), local NMDA application, and carotid clamping (Lin et al., 1995). These responses are mediated by the NMDAR because they are attenuated by NMDA antagonists (Kao et al., 1991; Lin et al., 1995, 1997).

Previous findings showed that blockade of the NMDAR by the nonselective antagonist dizocilpine (MK-801) or the GABA_A receptor by bicuculline (Jastrzebski et al., 1995) attenuated the hypotensive response to clonidine. These findings implicate the GABAergic and glutamatergic systems in the hypotensive action of clonidine and extended earlier findings that showed that clonidine enhances the spontaneous release of GABA, aspartate (Asp), and glutamate (Glu) in SHR, but not in Wistar-Kyoto rats (Tingley and Arneric, 1990). It is possible that the contribution of the glutamatergic system to the hypotensive action of clonidine depends on...
the ability of L-glutamate to release GABA because 1) stimulation of the excitatory amino acid receptor increases GABA release and facilitates the GABAergic synaptic activity (Petrovansky and Grantyn, 1990), and 2) blockade of the NMDAR decreases GABA outflow from the septum (Giovannini et al., 1994). These findings may explain the apparent paradox that both the inhibitory GABAergic and the excitatory glutamatergic systems contribute to clonidine-evoked hypotension. On the other hand, the possibility must be considered that L-glutamate, by virtue of its antagonistic effect of GABA-mediated response (Czyzewskaszafran et al., 1991; Gozlinska and Czyzewskaszafran, 1999) might attenuate the hypotensive effect of clonidine following the release of both neurotransmitters. In support of this notion is the pressor response elicited by L-glutamate or NMDA microinjection into the RVLM (Mao and Abdel-Rahman, 1995) and the abolition of the pressor response elicited by intra-RVLM NMDA in urethane-anesthetized rats by pretreatment with clonidine (Lin et al., 1997). Because clonidine acts on \( \alpha_2 \)-adrenergic and I1 receptors, we cannot ascertain from the previous findings (Lin et al., 1997) which receptor is involved in the interaction with the glutamate system, particularly with the NMDAR.

In this study we utilized the selective I1 receptor agonist rilmenidine to investigate the functional interaction between the I1 receptor and the NMDAR in the RVLM of the SHR. Nonetheless, because NMDA itself elicits a pressor response, we hypothesized that blockade of the NMDAR in the RVLM may enhance the hypotensive action of rilmenidine. An alternate, and attractive, hypothesis is that the I1 receptor-mediated response might be dependent on a novel inhibitory action of extrasynaptic NMDAR recently described in brain slices (Iaconson and Murphy, 2001). To address this question, we investigated the interaction between the I1 receptor and the NMDAR in the RVLM in vivo. To investigate whether NMDAR activation elicits an inhibitory action in our model system, NMDA was microinjected into the RVLM starting with approximately half the lowest dose used in reported studies (Lin et al., 1995; Mao and Abdel-Rahman, 1995), which consistently elicited pressor responses. Furthermore, the selective NMDAR antagonist AP5 was used to block the NMDAR in the RVLM in the current study, contrary to the systemic administration of the nonselective antagonist MK-801 in reported studies (Jastrzebski et al., 1995). AP5 is considered a highly selective and one of the most potent blockers of the NMDAR (Evans and Watkins, 1981; Childs et al., 1988). The present study utilized conscious SHRs to circumvent the potential confounding effects of anesthesia on the blood pressure responses elicited by rilmenidine.

### Materials and Methods

#### Preparation of the Rats

Male SHRs (Harlan, Indianapolis, IN), weighing 340 ± 30 g, 14 to 16 weeks old, were used in our experiments. All rats were housed in a room with controlled environment at a constant temperature of 23 ± 1°C, humidity of 50 ± 10%, and a 12-h light/dark cycle. Food and water were available ad libitum. Surgical procedures and postoperative care were performed in accordance with the Institutional Animal Care and Use Guidelines.

SHRs were anesthetized with methohexital sodium (Brevital, 50 mg/kg i.p.). The implantation of the guide cannula that allowed microinjection into the RVLM of conscious freely moving rats was performed as described in our previous studies (Mao and Abdel-Rahman, 1995). Briefly, the head of the animal was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and a 23-gauge stainless steel guide cannula was implanted unilaterally according to the following coordinates: rostro-caudal −12.8 mm, lateral 2.0 mm, vertical −8.0 mm relative to bregma, according to Paxinos and Watson (1982). The guide cannula was secured to the skull with dental cement and stainless steel screws. A stainless steel wire was used to seal the guide cannula until the day of the experiment. After 3 days, the animal was anesthetized with pentobarbital (50 mg/kg i.p.). A 5-cm PE-10 tube connected to PE-50 tubing filled with heparinized saline (heparin 200 units/ml) was placed in the abdominal aorta via the left femoral artery for measurement of blood pressure and heart rate, and the femoral vein catheter was used for the iv drug injections. The catheters were tunneled subcutaneously and exteriorized at the back of the neck between the scapulae, and plugged with stainless steel pins. Wounds were closed by surgical clips and swabbed with povidone-iodine solution. Each rat received similar volumes of ACSF in lieu of rilmenidine. Based on the dose-dependent hemodynamic responses to rilmenidine microinjected into RVLM.

#### Dose-Dependent Hemodynamic Response to Intra-RVLM Rilmenidine

Two groups of SHRs were used to investigate the role of the NMDA receptor in the hemodynamic responses to rilmenidine microinjected into RVLM.

A total of seven groups of conscious unrestrained SHRs (\( n = 5–10 \) each; Table 1) were used to investigate the role of the NMDA receptor in the hemodynamic responses to rilmenidine microinjected into RVLM. Cumulative doses of rilmenidine (10, 20, and 40 nmol) were unilaterally microinjected into RVLM at 10- to 15-min intervals. The control group received similar volumes of ACSF in lieu of rilmenidine. Based on the
findings of this experiment, 40 nmol of rilmenidine (80 nl) was utilized in subsequent studies.

Effects of AP5 on the Hypotensive Response Elicited by Intra-RVLM Rilmenidine. This experiment was designed to investigate the effect of the blockade of the RVLM NMDA receptor with AP5 on the hypotensive response elicited by rilmenidine. Two groups of SHRs received intra-RVLM AP5 (2 nmol, 40 nl) or ACSF (40 nl) 10 min before rilmenidine (40 nmol, 80 nl) microinjection into the same site, and the responses were followed for 40 min after rilmenidine. To further delineate the interaction between I<sub>L</sub> receptor and NMDA receptor in the RVLM, additional experiments were performed. The rats in the two additional groups received a microinjection of rilmenidine followed by AP5 (n = 5) or ACSF (n = 5).

Effect of NMDA Dose Level on Blood Pressure Response. In a pilot study, the effects of graded doses of intra-RVLM NMDA (10, 20, and 40 nmol) on blood pressure and heart rate were investigated. Whereas doses greater than 20 pmol of NMDA produced pressor responses (data not shown), which ruled out the possibility that the differences between the means of baseline MAP influenced the data interpretation.

Dose-Dependent Hypotensive Response Elicited by Microinjection of Rilmenidine. Cumulative doses (10, 20, and 40 nmol) of rilmenidine microinjected unilaterally at 10-min intervals into the RVLM of conscious SHRs caused dose-dependent hypotensive and bradycardic responses (Fig. 1). Notably, the 10-min interval may not have permitted the maximal hypotensive effect of each individual dose to be reached since it required more than 40 min for the maximal response to occur after a single dose of 40 nmol of rilmenidine (Fig. 2). This may explain the relatively modest hypotensive responses shown in Fig. 1. Microinjection of the same volume of ACSF had no effect on the blood pressure and heart rate (Fig. 1).

**TABLE 1** Baseline values of mean arterial pressure (mm Hg) and heart rate (HR, beats per minute)

<table>
<thead>
<tr>
<th>Groups</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSF (control)</td>
<td>163.2 ± 3.7</td>
<td>392 ± 20</td>
<td>5</td>
</tr>
<tr>
<td>Rilmenidine</td>
<td>152.3 ± 5.1</td>
<td>393 ± 11</td>
<td>10</td>
</tr>
<tr>
<td>Rilmenidine-AP5</td>
<td>171.0 ± 3.5</td>
<td>394 ± 26</td>
<td>5</td>
</tr>
<tr>
<td>AP5-Rilmenidine</td>
<td>154.3 ± 4.6</td>
<td>400 ± 32</td>
<td>5</td>
</tr>
<tr>
<td>Rilmenidine-ACSF</td>
<td>145.0 ± 7.8</td>
<td>396 ± 20</td>
<td>5</td>
</tr>
<tr>
<td>Rilmenidine-AP5</td>
<td>158.6 ± 5.7</td>
<td>386 ± 20</td>
<td>5</td>
</tr>
<tr>
<td>NMDA-AP5</td>
<td>159.8 ± 6.8</td>
<td>384 ± 25</td>
<td>8</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with the ACSF-rilmenidine group.

**Results**

The baseline values for blood pressure and heart rate were similar for the experimental and control groups (analysis of variance) except for a significant difference between the means of the baseline MAP of the ACSF-rilmenidine and AP5-rilmenidine groups (Table 1; t test). However, the difference between the standard deviations of these values was not significant (t test). Furthermore, comparison of the hypotensive responses elicited by rilmenidine in rats with relatively low or high baseline MAP revealed similar responses (data

![Fig. 1](image-url)
Effect of the NMDA Receptor Antagonist AP5 on the Hemodynamic Response to Rilmenidine.

Microinjection of AP5 (2 nmol) or ACSF did not change blood pressure or heart rate. However, pretreatment with AP5 abolished the hypotensive effect of rilmenidine (40 nmol). As shown in Fig. 2, intra-RVLM rilmenidine following ACSF caused gradual reductions in blood pressure and heart rate. However, AP5 pretreatment not only abolished the hypotensive response of rilmenidine but also changed it to a pressor response (17 ± 3.1 mm Hg, *P < 0.05; Fig. 2A). Pretreatment with AP5 also significantly (*P < 0.05) attenuated the bradycardic responses elicited by intra-RVLM rilmenidine (Fig. 2B).

Effect of Subsequent AP5 Microinjection on the Hypotensive Response Elicited by Intra-RVLM NMDA or Rilmenidine.

This experiment sought further evidence to support the dependence of the hypotensive action of rilmenidine on the RVLM NMDA receptor. To determine whether the blood pressure responses elicited by intra-RVLM NMDA were dose-dependent in the SHR, we investigated the effect of 10 to 40 pmol of NMDA on blood pressure and heart rate in a preliminary study. Whereas doses higher than 20 pmol elicited pressor responses (data not shown), a smaller dose of NMDA (10 pmol) produced hypotensive and bradycardic responses in conscious SHRs. The maximal reductions in MAP and heart rate elicited by 10 pmol of NMDA were 14.0 ± 3.1 mm Hg and 21 ± 12 beats/min, respectively. AP5 (2 nmol) counteracted the hypotensive response caused by NMDA and increased the blood pressure to levels higher than pre-NMDA values (Fig. 3A). A similar interaction was obtained between rilmenidine and AP5. The hypotensive and bradycardic responses elicited by intra-RVLM rilmenidine were counteracted by AP5, but not by an equal volume of ACSF (Fig. 3). Representative tracings depicting the hypotensive responses...
elicited by intra-RVLM rilmenidine or NMDA and their counteraction by AP5 are shown in Fig. 4. Histological verification of the site of injection indicated that the drug or vehicle injections were made into the RVLM in all rats from whom the data were collected (Fig. 5)

**Discussion**

Our present study presents two new findings. First, the hypotensive response elicited by the imidazoline (I₁) receptor in the RVLM is dependent on the NMDAR. Second, consistent with the recently described novel inhibitory action of extrasynaptic NMDAR in brain slices (Isaacson and Murphy, 2001), our findings reveal inhibitory action of NMDAR in the RVLM of conscious SHRs. To our knowledge, this is the first report of an inhibitory action of NMDAR in vivo, which results in hypotensive response.

The main objective of the present study was to obtain evidence in support of the hypothesis that the NMDAR in the RVLM plays a pivotal role in the hypotensive response caused by I₁ receptor activation. Previous studies, in anesthetized rats, showed that the selective I₁ agonist rilmenidine microinjected into the RVLM elicited a dose-dependent hypotensive and bradycardic responses (Ernsberger et al., 1990; Gomez et al., 1991). Our ability to replicate these reported findings in the conscious SHR made the latter an appropriate model for testing the stated hypothesis. We confirmed that the responses to rilmenidine in our experiments were elicited via its action on the RVLM neurons, based on chemical as well as histological verification of the site of injection. Notably, it is unlikely that the responses elicited by rilmenidine were mediated following its diffusion into other brainstem areas. Rilmenidine does not reduce blood pressure or heart rate when injected into the caudal ventrolateral medulla (CVLM) or the nucleus tractus solitarius (NTS) (Gomez et al., 1991).

Reported findings suggest that clonidine-evoked hypotension is closely related to the functional state of both the inhibitory GABAergic and the excitatory glutamatergic system (Jastrzebski et al., 1995). This evidence was based on the ability of GABA or NMDA antagonists to attenuate the hypotensive action of clonidine. Nonetheless, two main questions remained unanswered in these reported studies. First, it was not clear whether the reported interaction occurred between the NMDAR on the one hand and the I₁ or α₂ receptor on the other. In the reported studies, the mixed I₁/α₂ agonist clonidine was administered (Jastrzebski et al., 1995). Second, the drugs were administrated systematically (Jastrzebski et al., 1995), which makes it difficult to ascertain the neuroanatomical site of the interaction between clonidine and the glutamatergic system. It is also imperative to note that the systemic administration of MK-801, a nonselective NMDA receptor antagonist, causes an increase in blood pressure (Rockhold et al., 1992; Jastrzebski et al., 1995), which may confound the data interpretation. Even when we used a smaller dose than that reported, MK-801 still elicited a pressor response, that made it difficult to conclude whether the attenuation of the hypotensive effect of rilmenidine (i.v.) was a result of NMDAR blockade or the change in baseline blood pressure (data not shown). Furthermore, the use of anesthetics may have confounded the interpretation of the data in the reported studies (Jastrzebski et al., 1995).

We hypothesized that blockade of the NMDAR in the RVLM would enhance the hypotensive action of the selective I₁ agonist rilmenidine. The reasons for that assumption were: 1) clonidine enhances the release of L-glutamate from RVLM synaptosomes in SHRs (Tingley and Arneric, 1990), an effect that could be mediated by either the I₁ receptor or the α₂-receptor, and 2) the glutamatergic system within the RVLM plays an important excitatory role in the regulation of the arterial pressure. Reported findings including our own
have shown that intra-RVLM NMDA microinjection leads to pressor responses (Lin et al., 1995; Mao and Abdel-Rahman, 1995).

Contrary to our assumption, results of the present study showed that blockade of the NMDAR in the RVLM abolished the hypotensive response elicited by I1 receptor activation in the same area. Such a finding suggested that L-glutamate released upon the activation of the I1 receptor in the RVLM contributes to, rather than opposes, the hypotensive response. There are two possibilities that might explain this finding. First, the released L-glutamate may cause subsequent release of GABA within the RVLM, which is consistent with the increased release of both amino acids from RVLM neurons by clonidine (Tingley and Arneric, 1990). Stimulation of the excitatory amino acid receptor increases GABA release and facilitates the GABAergic synaptic activity (Perouansky and Grantyn, 1990), and blockade of the NMDAR decreases GABA outflow from the septum (Giovannini et al., 1994). It is known that activation of GABA receptor in the RVLM elicits hypotensive response (Jastrzubski et al., 1995). Second, a viable and appealing hypothesis is that L-glutamate released following I1 receptor activation acts, independent of the GABA pathway, through the NMDAR in the RVLM to produce the hypotensive response. This provocative hypothesis is supported by the recent discovery of a novel inhibitory role of the NMDAR in brain slices (Isaacson and Murphy, 2001). The present finding that NMDA microinjected into the RVLM of the conscious SHR elicited hypotensive response supports our alternate hypothesis. This is the first demonstration of a physiological relevance to the novel noninhibitory role of the NMDAR, recently described in brain slices (Isaacson and Murphy, 2001). In both studies, the NMDAR-mediated inhibitory responses, the extrasynaptic inhibition (Isaacson and Murphy, 2001), and the hypotensive response (this study) were blocked by the selective NMDAR antagonist AP5. Nonetheless, other alternative explanations must be considered. For example, rilmenidine may act directly or indirectly to elicit the hypotensive response as well as the pressor response that becomes unmasked by the NMDAR blockade. As discussed earlier, clonidine (mixed I1 and α2 agonist) enhances the release of L-glutamate and GABA from RVLM neurons (Tingley and Arneric, 1990). It is also possible that rilmenidine provokes the release of inhibitory and excitatory neuromodulators other than glutamate and GABA or interacts with binding site(s) other than the NMDAR. In support of these possibilities are the findings that the imidazol(di)ne compounds interact with the phenycyclidine-binding site on the NMDAR (Olmos et al., 1996). However, since other receptors/channels, such as the nicotinic acetylcholine receptor and the K+ channel, share with the NMDAR the phenycyclidine-binding site (Olmos et al., 1996), it is possible that rilmenidine produces its actions through the interaction with one or more of these sites.

It is important to comment on the role of the NMDA dose as well as the model system employed in the hypotensive response elicited by the NMDAR in the RVLM. Doses of NMDA (>20 pmol) microinjected into the RVLM elicited the expected pressor response, which agrees with reported findings in conscious or anesthetized normotensive rats (Mao and Abdel-Rahman, 1994; Lin et al., 1997). Nonetheless, a smaller dose (10 pmol) of NMDA, which constitutes 25 to 50% of the lowest doses employed in reported studies, elicited a hypotensive response in the conscious SHR. It is possible that the hypotensive results from the activation of the NMDAR in the RVLM of an animal model known to exhibit enhanced RVLM neuronal activity (Lin et al., 1995). The hypotensive response, in our model system, is NMDAR-mediated because the response was attenuated by the selective NMDAR antagonist AP5. The dose of AP5 employed in the present study adequately blocked the NMDAR-mediated responses in reported studies including our own (Kubo et al., 1993; Mao and Abdel-Rahman, 1995). Notably, the same dose of AP5 also attenuated the hypotensive response elicited by I1 receptor activation in the RVLM of the conscious SHR. Together, the present findings provide correlative evidence that supports a pivotal role for the NMDAR in the RVLM in the hypotensive response elicited by I1 receptor activation in the same brain area. Notably, AP5 microinjection into the RVLM, as in other studies (Mao and Abdel-Rahman, 1995), did not change baseline blood pressure and, therefore, circumvented the confounding effect of an increase in blood pressure, caused by systemic MK-801 in reported studies (Jastrzubski et al., 1995) and in our pilot study, on data interpretation. Finally, the hypotensive response elicited by NMDA does not seem to be a result of its leakage from the RVLM into the CVLM or the NTS. Histological verification revealed the microinjection sites to be confined to the RVLM.

We conclude that in the conscious SHR, the activation by rilmenidine of the I1-imidazoline receptor in the RVLM elicits hypotension that is dependent on functional NMDAR. Furthermore, the present study describes a noninhibitory role for the NMDAR in the RVLM, triggered by small amounts of NMDA, and leads to hypotensive response in the conscious SHR. Finally, the present findings demonstrate, for the first time, that a common binding site on the NMDAR recognized by NMDA and I1 receptor agonists, identified in vitro (Olmos et al., 1996), plays a functional role in vivo, and may contribute to the hypotensive action of the second generation centrally acting drugs.

References


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