Augmented Responses to Intrathecal Nicotinic Agonists in Spontaneous Hypertension

Imran M. Khan, Morton P. Printz, Tony L. Yaksh, Palmer Taylor

Abstract Abnormal central cholinergic activity has been reported to be responsible in part for the pathogenesis of high blood pressure in spontaneously hypertensive rats (SHR). Administration of cholinergic agonists in brain and spinal cord results in exaggerated pressor responses in SHR. Studies to date have focused largely on the muscarinic cholinergic system. Recently, we demonstrated that intrathecal administration of nicotinic agonists results in pressor, tachycardic, and irritation responses. In the present study we examine the cardiovascular and behavioral responses to nicotine and cytisine administered intrathecally in La Jolla strain (LJ) SHR LJ and age-matched Wistar-Kyoto (WKY LJ) rats. Nicotinic agonists produced augmented pressor, heart rate, and irritation responses in SHR LJ compared with normotensive rats. In both SHR LJ and WKY LJ rats, cytisine elicited a greater nociceptive response and greater spinobulbar component to the pressor response than nicotine. SHR LJ and WKY LJ rats also differ in that the SHR LJ strain shows a diminished tendency for desensitization to cytisine. As in Sprague-Dawley rats, in SHR LJ and WKY LJ rats the cardiovascular and behavioral responses to intrathecal nicotine were significantly inhibited by mecamylamine, dihydro-β-erythroidine, and methyllycaconitine. However, methyllycaconitine, which effectively blocked cytisine-elicited cardiovascular and behavioral responses in Sprague-Dawley and WKY LJ rats, was unable to inhibit the maximal rise in cytisine-elicited blood pressure, heart rate, and irritation responses in SHR LJ. In contrast to the heightened cardiovascular and behavioral responses, the number of nicotinic binding sites in spinal cord membranes was significantly decreased in the hypertensive rats. The exaggerated responses to spinal nicotinic agonists in the presence of lower receptor number and the lower propensity to desensitize to cytisine-elicited irritation responses in SHR LJ suggest that amplification of postcoupling events is enhanced in the hypertensive rats. (Hypertension. 1994;24:611-619.)

Key Words • nicotine • receptors, nicotinic • receptors, cholinergic • rats, inbred SHR

The central cholinergic nervous system has been implicated in the pathogenesis of hypertension in several rat models, including the spontaneously hypertensive rat (SHR).1-4 Central administration of cholinergic agonists leads to increases in blood pressure and heart rate in SHR and normotensive rats; however, the responses are augmented in SHR.5-8 Inhibition of the central cholinergic system in young SHR can delay the onset of hypertension1,2 or cause a fall in blood pressure in mature hypertensive rats.8 The exaggerated cardiovascular responses to cholinergic agonists in SHR compared with normotensive rats can be observed at both spinal6 and supraspinal5,12 levels. In the brain, a role of muscarinic receptors in the maintenance of high blood pressure in hypertensive rats is evident.9,10 On the other hand, several studies have shown that nicotinic receptor stimulation in lower brain stem leads to a depressor response; also, the number of nicotinic binding sites in these regions is reported to be lower in SHR compared with normotensive rats.11,12 However, in a recent study Tseng et al.13 demonstrated that microinjections of nicotine in the rostral ventrolateral medulla produce an augmented pressor and tachycardic response in SHR compared with Wistar-Kyoto (WKY) or Sprague-Dawley (SD) normotensive rats.

Similar to their role in brain, a role of muscarinic receptors in mediating an exaggerated pressor response in SHR has been demonstrated in the spinal cord.6 However, detailed studies of spinal nicotinic acetylcholine receptors have not been conducted. We previously observed that intrathecal administration of nicotinic agonists causes an increase in blood pressure and heart rate and results in nociceptive behavior.13 The cardiovascular and behavioral responses appear to be caused by a localized spinal action of the nicotinic agents, and the cardiovascular response results from an increase in spinal sympathetic outflow.14 Moreover, nicotinic agonist-elicited blood pressure and behavioral responses occur via independent receptor-linked pathways. Finally, nicotinic receptor binding can be identified in regional areas of the spinal cord, and the locations of binding sites correlated with functional pharmacological end points.15

In the present study we document the cardiovascular and behavioral responses to spinal administration of nicotinic agonists in La Jolla strain (LJ) SHR LJ and compare them with those found in WKY LJ rats. Additionally, we compare the pharmacological specificity of the spinal nicotinic receptors in both SHR LJ and WKY LJ rats. Finally, we ascertain ligand binding to nicotinic receptors in the spinal cord membranes of both rat strains to determine whether the number or affinity of the nicotinic receptors differs between SHR LJ and WKY LJ rats.

Methods

Experimental Animals

Male rats used for cardiovascular and behavioral testing were age matched (12 to 16 weeks old). All animals were bred.
and maintained (12-hour diurnal cycle) within our breeding colony located within the animal care facility at the University of California, San Diego (UCSD). WKY rats were from stock originally obtained from Charles River Laboratories (Wilmington, Mass) and were brother-sister mated through 18 or more generations. SHR were also derived from Charles River Laboratories and maintained by brother-sister mating. Since these animals have been bred in La Jolla since 1981, they are referred to as SHR\(_{\text{LJ}}\) and WKY\(_{\text{LJ}}\). All studies were carried out according to protocols approved by the UCSD Institutional Animal Care Committee.

**Cardiovascular and Behavioral Studies**

For spinal drug delivery, intrathecal catheters were implanted as described previously.\(^6\) Briefly, rats were anesthetized with halothane (2% to 3%), and 9-cm saline-filled PE-10 tubing was placed into the intrathecal space through the cisterna membrane. The catheter tip resided at the rostral end of the lumbar enlargement.\(^12\) The catheter was externalized on top of the skull, and the incision was closed. Rats were allowed to recover for at least 4 days before further study, and only animals exhibiting normal motor behavior were used in the study.

Rats with intrathecal implants were catheterized in the tail artery with PE-50 tubing under halothane (2% to 3%) anesthesia as described previously.\(^12\) Heart rates were measured with a cardiotachometer triggered from the pressure pulses. Blood pressure and heart rate were recorded on a polygraph (model 7, Grass Instrument Co).

Behavioral responses were measured according to a scoring protocol described earlier.\(^12\) Briefly, a score of 1 was given for each of the following behavioral responses: movement of the limbs, twisting and turning, tail erection, and high-pitched squeaking. The maximum assignable score was 4.

Drugs were dissolved in sterile saline solutions and administered with a hand-driven syringe pump over a period of 15 to 25 seconds. All drug solutions were prepared to achieve a 10-μL final delivery volume. Drug administration was followed by 10 μL of saline flush within 15 seconds to clear the catheter of drugs.

**Experimental Paradigms for Drug Administration**

1. **Agonist Dose-Response Relations.** Dose-response curves for agonists were developed by administration of agonists at 10-fold increases in dose every 25 to 35 minutes. During this time the three parameters measured always returned to baseline.

2. **Agonist Desensitization.** After the administration of the highest dose in paradigm 1, desensitization to agonists for the three parameters was evaluated by repeated administration of the highest dose at 25- to 35-minute intervals.

3. **Antagonist Specificity to Spinal Nicotinic Agonist-Elicited Responses.** The effect of a near maximal dose (50 μg IT) of the antagonist, determined previously,\(^12\) was used to block the response elicited by 5 μg of agonist. Since cytisine showed desensitization, antagonism of cytisine responses was determined with separate rats and compared with vehicle-treated rats receiving cytisine only.

**Spinal Cord Membrane Preparation**

Membranes were prepared according to the procedure described earlier.\(^12\) Briefly, a 5-cm (1 cm from the sacral end) segment of the spinal cord was dissected and placed in a polypropylene tube. For measurement of \(^3\)H-cytisine binding in the dorsal lumbar and ventral lumbar regions and the intermediolateral spinal cord, the 5-cm spinal cord segment was dissected into a 2-cm segment of lumbarosacral and 3-cm segment of intermediolateral spinal cord. The lumbarosacral spinal cord was dissected into dorsal and ventral portions. All tissue sections were stored at −70°C.

Spinal cord sections were homogenized in ice-cold 50 mmol/L Tris-HCl buffer, pH 7.4.\(^15\) The homogenate was centrifuged at 48,000g for 10 minutes; the pellet was resuspended in fresh buffer and centrifuged a second time; and the final pellet was resuspended in fresh buffer.

**Equilibrium Binding Assays**

Equilibrium binding assays were conducted according to the procedures described earlier.\(^15\) Briefly, the assay mixture consisted of 400 to 600 μg of membrane protein in a final incubation volume of 125 μL. Final concentrations of \(^3\)H-cytisine varied between 0.05 and 10 nmol/L (250 to 50,000 cpm); stock solutions were prepared in assay buffer. Incubations were carried out in a cold room (4°C) with gentle shaking for 60 minutes. Assays were initiated by the addition of the membrane suspension with rapid mixing to the \(^3\)H-cytisine solutions in a polypropylene tube. The incubations were terminated by dilution with 3 mL ice-cold assay buffer immediately followed by rapid filtration under vacuum through Whatman GF/C filter papers previously equilibrated with 0.5% polyethyleneimine at 4°C. Filters were then rinsed three times with 3 mL of ice-cold buffer. Specific binding was determined as the difference in binding between samples containing excess unlabeled l-nicotine (40 μmol/L) and those containing only \(^3\)H-cytisine. Protein was assayed by the biocinchoninic acid protein assay.

**Drugs**

The following chemicals were obtained from Sigma Chemical Co: l-nicotine, cytisine, atropine sulfate, and mecamylamine. Methyllycaconitine (MLA) and dihydro-/β-erythroidine (DβE) were from Research Biochemicals International.

**Statistics**

All values presented are mean±SEM. Student’s t test for unpaired data was used to determine differences between two treatment groups. Differences between multiple groups were compared using ANOVA.

**Results**

**Basal Systolic Blood Pressure and Heart Rate in SHR\(_{\text{LJ}}\) and WKY\(_{\text{LJ}}\) Rats**

Basal systolic blood pressure before each pharmacological intervention was significantly higher in SHR\(_{\text{LJ}}\) (194±2 mm Hg, n=54) than in age-matched WKY\(_{\text{LJ}}\) littermates (139±1 mm Hg, n=49, P<.0001) over the course of this study. Similarly, basal heart rate was slightly but significantly elevated in SHR\(_{\text{LJ}}\) (464±5 beats per minute [bpm]) compared with WKY\(_{\text{LJ}}\) rats (446±4 bpm, P<.007). In the animal subgroups used in the different experimental paradigms, systolic blood pressure was always significantly higher in SHR\(_{\text{LJ}}\) than in age-matched WKY\(_{\text{LJ}}\) rats. However, heart rate was not significantly elevated in SHR\(_{\text{LJ}}\) over age-matched WKY\(_{\text{LJ}}\) rats; greater significance might be achieved with a larger sample size.

**Cardiovascular and Behavioral Responses to Spinal Nicotinic Agonists in SHR\(_{\text{LJ}}\) and WKY\(_{\text{LJ}}\) Rats**

Basal systolic blood pressures and heart rates in SHR\(_{\text{LJ}}\) (n=19) and WKY\(_{\text{LJ}}\) rats (n=17) used for this protocol were 190±2 and 136±2 mm Hg (P<.001) and 478±8 and 453±7 bpm (P<.02), respectively.

Intrathecal nicotine produced a dose-dependent increase in blood pressure, heart rate, and irritation responses in both SHR\(_{\text{LJ}}\) and WKY\(_{\text{LJ}}\) rats; however, the nicotine-elicited rises in blood pressure and heart rate
Nicotine (nmoles, i.t.)

FIG 1. Line graphs show changes in systolic blood pressure (SBP), heart rate, and irritation index after intrathecal injection of 0.05, 0.5, and 5.0 \( \mu \text{g} \) \((-\)-nicotine in spontaneously hypertensive (\( \square \), \( n=10 \)) and Wistar-Kyoto (\( \Delta \), \( n=8 \)) rats. Responses of SBP and heart rate to 5.0 \( \mu \text{g} \) IT nicotine and irritation responses to 0.5 \( \mu \text{g} \) IT nicotine, respectively, in Sprague-Dawley rats (\( \odot \), \( n=6 \)) are also shown.\(^1\) Each value represents mean±SEM. *\( P<0.001 \), \( \dagger P<0.01 \), \( \ddagger P<0.05 \).

were greater in SHR\(_{LJ}\) compared with WKY\(_{LJ}\) rats, with the largest difference observed at 11 nmol (5 \( \mu \text{g} \)) of administered nicotine (Fig 1A and 1B). The pressor and heart rate responses elicited by nicotine were also significantly higher than those found in SD rats (also at 5 \( \mu \text{g} \) nicotine).\(^1\)

In contrast to the cardiovascular responses, the irritation response to spinal nicotine did not differ between the three strains at higher doses of the agonist (Fig 1C). However, SHR\(_{LJ}\) were more sensitive than the normotensive controls in producing nicotine-elicited behavioral responses at the lowest dose of 0.05 \( \mu \text{g} \). Although all three rat strains exhibited maximal irritation scores of 4 at the highest drug dose, the irritation response in SHR\(_{LJ}\) appeared most intense to the evaluator.

Similar to nicotine, intrathecal cytisine also elicited augmented pressor responses in SHR\(_{LJ}\) compared with normotensive rats (Fig 2A); however, the largest difference was observed between SHR\(_{LJ}\) and normotensive rats at 2.6 nmol (0.5 \( \mu \text{g} \)) cytisine. Although in our previous study a leveling off of the dose dependence in pressor response in SD rats was not observed, in the present study the maximal attainable increase in systolic blood pressure was observed by 0.5 \( \mu \text{g} \) cytisine in both SHR\(_{LJ}\) and WKY\(_{LJ}\) rats (Fig 2A). The heart rate response to cytisine in SHR\(_{LJ}\) was not greater than in WKY\(_{LJ}\) rats (Fig 2B). SHR\(_{LJ}\) were more sensitive to the cytisine-elicited nociceptive response, similar to the nicotine responses, than either WKY or SD rats at the lowest dose (0.05 \( \mu \text{g} \)) (Fig 2C).

Effects of Repeated Intrathecal Administration of Nicotine and Cytisine on Cardiovascular and Behavioral Responses in SHR\(_{LJ}\) and WKY\(_{LJ}\) Rats

We previously demonstrated\(^1\) that in SD rats sequential administrations of nicotine did not cause appreciable desensitization to the agonist-elicited responses. As in SD rats, repeated administrations of intrathecal nicotine in both SHR\(_{LJ}\) and WKY\(_{LJ}\) rats did not cause a significant decrease in the magnitude of any of the responses (data not shown). However, repeated administrations of cytisine revealed a marked decrease in the magnitude of the responses in SD rats,\(^1\) and in the present study both SHR\(_{LJ}\) and WKY\(_{LJ}\) rats exhibited desensitization of all three responses to repeated dosing of cytisine. The propensity for desensitization of the three responses, particularly the irritation response, was diminished in the SHR\(_{LJ}\) strain compared with normotensive strains. There was a marked reduction in the magnitude of the three responses in all rat groups with the second dose of 26 nmol (5 \( \mu \text{g} \)) of intrathecal cytisine (Fig 3A through 3C). However, on the third dose, the two normotensive strains...
obtained in SD rats for nicotine and the three nicotinic receptor antagonists. None of the antagonists lowered
were also significantly higher in SHR LJ (Fig 3A and 3B). Similarly, the com-
channel blocker mecamylamine almost completely abol-
petitive nicotinic antagonist D/3E also significantly inhib-
strains showed further blunting of the cytisine-elicited
ions in both SHR LJ and WKY LJ rats (Table 1). Likewise, MLA, which appeared to exhibit mixed
competitive and noncompetitive (or channel) block-
strains showed further blunting of the cytisine-elicited
response to cytisine in SHR LJ was significantly higher than that in WKY LJ and SD rats; however, the score of 4 for the irritation response was already evident by the first minute (Fig 4A through 4C). Thus, in SHR LJ, the irritation response, by virtue of its more rapid onset, may also contribute to the increased pressor response in the first minute.
In WKYLJ rats, DβE blocked the tachycardia only in the second minute after cytisine (Fig 4E). The onset of the irritation response, similar to that in SD rats (unpublished observations), was delayed until the second minute (Fig 4F), and DβE blocked only the rise in irritation occurring in the second minute. DβE had no effect on the pressor response to intrathecal cytisine in WKYLJ rats (Fig 4D). It appears that the initial pressor response to cytisine in WKYLJ rats is less sensitive to DβE than in SHR LJ and SD rats.
Similar to its effect on cytisine-elicited responses in SD rats, MLA significantly inhibited the cardiovascular and behavioral responses to cytisine in WKYLJ rats (Table 2, Fig 5D through 5F). However, cytisine-elicited maximal increases in pressor, heart rate, and irritation responses in SHR LJ were not blocked by MLA at this dose. Although the maximal rises in blood pressure, heart rate, and irritation responses to intrathecal cytisine were not blocked in SHR LJ, MLA significantly inhibited the rise in blood pressure until the second minute and the rise in heart rate and the irritation response up until the third minute after cytisine administration (Fig 5A through 5C). The data indicate that MLA exhibits a slightly different pharmacological specificity in blocking spinal nicotinic receptors in SHR LJ compared with SD or WKYLJ rats.

**Effect of Nicotinic Receptor Antagonists on Nicotine-Elicited Cardiovascular and Behavioral Responses in SHR LJ and WKY LJ Rats**

Basal systolic blood pressures and heart rates in SHR LJ (n=17) and WKY LJ rats (n=14) used for this protocol were 194±3 and 141±2 mm Hg (P<.001) and 464±9 and 451±6 bpm (P>.05), respectively. The ion channel blocker mecamylamine almost completely abolished the three responses to intrathecal nicotine in both SHR LJ and WKY LJ rats (Table 1). Similarly, the competitive nicotinic antagonist DβE also significantly inhibited the responses to nicotine in both rat strains (Table 1). Likewise, MLA, which appeared to exhibit mixed competitive and noncompetitive (or channel) blockade, also significantly blocked the cardiovascular and behavioral responses to nicotine in the two rat groups (Table 1). The data were essentially similar to those obtained in SD rats for nicotine and the three nicotinic receptor antagonists. None of the antagonists lowered
blood pressure or heart rate in SHR LJ or WKY LJ rats (data not shown).

**DβE and MLA Antagonism of Intrathecal Cytisine-Elicited Responses in SHR LJ and WKY LJ Rats**

Basal systolic blood pressures and heart rates in SHR LJ (n=17) and WKY LJ rats (n=17) used for this protocol were 197±3 and 140±2 mm Hg (P<.001) and 449±9 and 434±7 bpm (P>.05), respectively.

Prior treatment with DβE did not affect the maximal cytisine-elicited responses (within 1 to 4 minutes after cytisine administration) in SHR LJ or WKY LJ rats (Table 2). However, similar to the situation observed in SD rats, this competitive antagonist significantly blocked the pressor and heart rate responses to intrathecal cytisine for the first minute of the response after agonist administration (Fig 4A and 4B) in SHR LJ. In addition, complete manifestation of cytisine-induced nociceptive response after DβE was also delayed to the third minute compared with untreated SHR LJ (Fig 4C). In SHR LJ, without antagonist, the pattern of cytisine-elicited rise in blood pressure and heart rate was similar to that in SD rats; however, the score of 4 for the irritation response was already evident by the first minute (Fig 4A through 4C). Thus, in SHR LJ, the irritation response, by virtue of its more rapid onset, may also contribute to the increased pressor response in the first minute.

In WKY LJ rats, DβE blocked the tachycardia only in the second minute after cytisine (Fig 4E). The onset of the irritation response, similar to that in SD rats (unpublished observations), was delayed until the second minute (Fig 4F), and DβE blocked only the rise in irritation occurring in the second minute. DβE had no effect on the pressor response to intrathecal cytisine in WKY LJ rats (Fig 4D). It appears that the initial pressor response to cytisine in WKY LJ rats is less sensitive to DβE than in SHR LJ and SD rats.

Similar to its effect on cytisine-elicited responses in SD rats, MLA significantly inhibited the cardiovascular and behavioral responses to cytisine in WKY LJ rats (Table 2, Fig 5D through 5F). However, cytisine-elicited maximal increases in pressor, heart rate, and irritation responses in SHR LJ were not blocked by MLA at this dose. Although the maximal rises in blood pressure, heart rate, and irritation responses to intrathecal cytisine were not blocked in SHR LJ, MLA significantly inhibited the rise in blood pressure until the second minute and the rise in heart rate and the irritation response up until the third minute after cytisine administration (Fig 5A through 5C). The data indicate that MLA exhibits a slightly different pharmacological specificity in blocking spinal nicotinic receptors in SHR LJ compared with SD or WKY LJ rats.

**[3H]Cytisine Binding in Spinal Cord Membranes of SHR LJ and WKY LJ Rats**

As shown in Fig 6, [3H]cytisine showed saturable binding to a single class of binding sites with no cooperativity in spinal cord membranes of both SHR LJ and WKY LJ rats. Although the affinity of [3H]cytisine for the binding sites did not differ between the two rat strains, the total number of sites was higher in the normotensive WKY LJ rats. The total number of binding sites in the whole spinal cord (5-cm segment) in WKY LJ rats did not differ significantly from that in SD rats.
The total number and affinity of the nicotinic receptors in various regions of the spinal cord were also determined in SHR_LJ and WKY_LJ rats (Table 3). The affinity of the receptors did not differ significantly between strains. The larger number of binding sites was found in the dorsal lumbarosacral region followed by the intermediolateral region in both SHR_LJ and WKY_LJ rats. The lowest number of binding sites was observed in the ventral lumbarosacral region (Table 3). Similar to the whole spinal cord, fewer binding sites were found in SHR_LJ than in WKY_LJ rats in all three regions. No difference in protein concentration in any of the spinal cord regions was observed between the two rat groups (data not shown).

**Discussion**

Intrathecal administration of nicotine and cytisine elicits dose-dependent increases in blood pressure and heart rate and results in the manifestation of nociceptive responses in both SHR_LJ and age-matched normotensive WKY_LJ rats. These qualitative observations are similar to those we reported for SD rats.13 The pressor and heart rate responses to nicotine and the pressor response to cytisine were exaggerated in SHR_LJ compared with either WKY_LJ or SD normotensive rats. Although SHR_LJ did not show a potentiated nociceptive response to either nicotine or cytisine at the highest dose used (5 μg), the behavioral response was greater in SHR_LJ at a 0.05-μg dose of either agonist compared with both normotensive rat strains. As in SD rats, the duration of action of cytisine was longer than nicotine in both SHR_LJ and WKY_LJ rats.

Cholinergic components of the central nervous system have been implicated in the regulation of cardiovascular and behavioral responses. Hyperactivity in this compo-

**TABLE 3. Effects of Mecamylamine, Dihydro-β-erythroidine, and Methyllycaconitine on Maximal Pressor, Heart Rate, and Behavioral Responses Induced by 5 μg IT Nicotine in Conscious SHR_LJ and WKY_LJ Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Response Remaining in SHR_LJ, %</th>
<th>Response Remaining in WKY_LJ Rats, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>7±3 (n=5)*</td>
<td>8±5 (n=5)†</td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>22±10 (n=7)†</td>
<td>26±11 (n=5)†</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>24±13 (n=5)§</td>
<td>13±4 (n=4)§</td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>21±14§</td>
<td>11±8‡</td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>21±14∥</td>
<td>6±9‡</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>19±6∥</td>
<td>36±13∥</td>
</tr>
<tr>
<td>Irritation index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>5±5*</td>
<td>20±12†</td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>27±10†</td>
<td>13±10†</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>35±13∥</td>
<td>44±12§</td>
</tr>
</tbody>
</table>

SHR_LJ indicates spontaneously hypertensive rats, La Jolla strain; WKY_LJ, Wistar-Kyoto rats, La Jolla strain. Each value represents percentage of response compared with responses before antagonist administration (each 50 μg IT).

*P<.0001, †P<.0003, ‡P<.001, §P<.004, ||P<.03 compared with saline-pretreated rats.

**TABLE 2. Effects of Dihydro-β-erythroidine and Methyllycaconitine on Maximal Pressor, Heart Rate, and Behavioral Responses Induced by 5 μg IT Cytisine in Conscious SHR_LJ and WKY_LJ Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Response Remaining in SHR_LJ, %</th>
<th>Response Remaining in WKY_LJ Rats, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>79±9 (n=5)</td>
<td>88±11 (n=6)</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>90±9 (n=5)</td>
<td>51±11 (n=5)*</td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>91±19</td>
<td>84±11</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>72±14</td>
<td>54±13†</td>
</tr>
<tr>
<td>Irritation index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>100</td>
<td>53±8‡</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1. Each value represents percentage of response compared with responses in the absence of antagonist (each 50 μg IT).

*P<.01, †P<.015, ‡P<.001 compared with saline-pretreated rats.
Fig. 4. Line graphs show dihydro-β-erythroidine antagonism of cytisine-elicited systolic blood pressure (SBP) (A and D), heart rate (B and E), and behavioral (C and F) responses in spontaneously hypertensive rats (SHR J) and Wistar-Kyoto (WKY J) rats, respectively. Closed and open circles represent control (n=7) and treated (n=5) SHR J rats; closed and open triangles represent control (n=5) and treated (n=6) WKY J rats, respectively. Horizontal axis represents values measured during the first, second, and third minutes after a single intrathecal administration of cytisine (5 μg). Each value represents mean±SEM. *P<.006, tP<.008, §P<.02,  HP<.05 compared with control responses at the respective time points.

Fig 5. Line graphs show methyllycaconitine antagonism of cytisine-elicited systolic blood pressure (SBP) (A and D), heart rate (B and E), and behavioral (C and F) responses in spontaneously hypertensive rats (SHR J) and Wistar-Kyoto (WKY J) rats, respectively. Closed and open circles represent control (n=7) and treated (n=5) SHR J rats; closed and open triangles represent control (n=5) and treated (n=6) WKY J rats, respectively. Horizontal axis represents values measured during the first, second, and third minutes after a single intrathecal administration of cytisine (5 μg). Each value represents mean±SEM. *P<.002, †P<.003, §P<.001, ‡P<.01, #P<.05 compared with control responses at the respective time points.
The delayed pressor response of longer duration from spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats. This finding may relate to the role of muscarinic cholinergic receptors. Our study shows that nicotinic cholinergic receptor stimulation in the spinal cord also results in an augmented cardiovascular response in a hypertensive rat model. In addition, our study establishes that spinal nicotinic receptor stimulation leads to a potentiated nociceptive response. This may indicate that spinal nicotinic receptors are involved in the pathogenesis of hypertension in SHR.

We have shown previously that the pressor and behavioral responses elicited by nicotinic agonists are mediated through two distinctly located and independent receptor-linked pathways. Nicotine and cytisine show differential stimulatory capacities for pressor and nociceptive responses. The major portion of the pressor response to nicotine is mediated by nicotinic receptor stimulation in the thoracic spinal cord of sympathetic output, whereas the nociceptive response to spinal nicotine requires an intact bulbo-sacral pathway. The cytisine-elicited pressor response, on the other hand, has two components: an initial transient fast desensitizing response (probably resulting from nicotinic receptor stimulation in the intermediolateral region) and a secondary delayed pressor response of longer duration associated with the onset of the irritation or nociceptive response. This component is mediated through the spinobulbar pathway. Moreover, the irritation response to spinal cytisine, in contrast to nicotine, is not sensitive to competitive nicotinic receptor antagonists. Although the heart rate response is variable, in most experimental conditions the increased heart rate appears to correlate with the irritation response.

As in SD rats, repetitive intrathecal administration of nicotine in both SHR and WKY rats did not reveal desensitization of the pressor, heart rate, and nociceptive responses. However, with repetitive cytisine administration, the three rat strains exhibited desensitization. Of the three strains, the SHR showed a diminished propensity to desensitize. Reduced desensitization was most evident with the irritation response.

In addition to the slow rate of desensitization to cytisine-elicited responses, SHR showed a very rapid onset of cardiovascular and behavioral responses to intrathecal cytisine. The maximal rise in blood pressure, heart rate, and nociceptive responses occurred within the first minute after cytisine administration (Fig 4A through 4C). However, in WKY rats, the onset of the maximal rise in blood pressure, heart rate, and irritation responses were delayed (Fig 4D through 4F). Interestingly, SD rats showed onset profiles to cytisine-elicited pressor and heart rate responses similar to those in SHR but showed a delayed onset of the irritation response. Thus, SHR show different rates of onset and desensitization of responses to cytisine than SD or WKY rats.

Nicotinic receptor antagonists exhibited inhibitory profiles to nicotine in SHR and WKY rats resembling those in SD rats. However, the competitive nicotinic antagonists D/3E and MLA produced interesting differences for antagonism of cytisine-elicited responses in these two rat groups. In contrast to the responses in SD and SHR rats, in WKY rats D/3E did not block the initial rise in blood pressure after intrathecal cytisine administration. This may indicate that D/3E sensitivity for spinal nicotinic receptors may differ in WKY rats. Alternatively, as shown in the dose-response curve for cytisine in WKY rats (Fig 2A), the maximal pressor response may be saturated by the 0.5-μg dose of cytisine. This may also be true for the initial pressor response to intrathecal cytisine. Thus, the inhibitory effect of D/3E may not be as apparent with a 5-μg dose of cytisine, because its response lies near the maximal plateau of the cytisine dose-response curve. D/3E blocked the initial rise in irritation response in both SHR and WKY rats, suggesting that cytisine and

### Table 3. Equilibrium Dissociation Constants \( K_d \) and Total Saturable Sites \( B_{max} \) of \[^3H\]Cytisine Binding to Membrane Preparations From Various Spinal Cord Regions of SHR and WKY Rats

<table>
<thead>
<tr>
<th>Spinal Cord Region</th>
<th>SHR ( K_d )</th>
<th>WKY ( K_d )</th>
<th>SHR ( B_{max} )</th>
<th>WKY ( B_{max} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole (5-cm) segment</td>
<td>0.70±0.14</td>
<td>0.59±0.12</td>
<td>13.1±0.1</td>
<td>17.2±1.2</td>
</tr>
<tr>
<td>Dorsal lumbarosacral</td>
<td>1.08±0.34</td>
<td>1.05±0.34</td>
<td>18.1±0.9</td>
<td>22.4±0.9</td>
</tr>
<tr>
<td>Ventral lumbarosacral</td>
<td>0.85±0.10</td>
<td>0.70±0.20</td>
<td>11.5±0.5</td>
<td>14.1±0.6</td>
</tr>
<tr>
<td>Intermediolateral cell column</td>
<td>0.82±0.30</td>
<td>0.66±0.20</td>
<td>12.9±0.4</td>
<td>16.0±0.7</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1. Standard error represents three to four measurements on separate preparations. Each preparation was made from pooled spinal cord membranes from six to nine rats.

\*P<.01, \*\*P<.02, \*\*\*P<.04 compared with SHR for the same spinal cord region.
nicotine share a common mechanism for eliciting the initial portion of the irritation response.

In contrast to DβE, MLA blocked significantly all three responses to intrathecal cytisine in WKY\textsubscript{LJ} rats. In SHR\textsubscript{LJ}, MLA did not block the maximal rise in blood pressure, heart rate, and irritation responses. However, it significantly inhibited the initial rise in blood pressure and heart rate responses after cytisine administration. In addition, it delayed manifestation of the irritation response in the first 2 minutes after cytisine administration. Thus, MLA antagonizes a different complement of nicotinic receptors in the spinal cord.

Interestingly, none of the nicotinic receptor antagonists had any effect on basal heart rate and blood pressure in SHR\textsubscript{LJ} or normotensive rats, suggesting that spinal nicotinic receptors may not have a dominant role in maintaining higher basal blood pressure in SHR\textsubscript{LJ}. It is well documented that sympathetic nerve activity is elevated in SHR compared with WKY rats.\textsuperscript{20,22} In addition, the baroreceptor reflex is blunted in SHR.\textsuperscript{23} Although altered sympathetic activity of central nervous system origin is known to be involved in the pathogenesis of hypertension in SHR, the blunted baroreceptor activity in SHR compared with WKY rats is evident after the onset of hypertension in SHR.\textsuperscript{21,22} As already mentioned, intrathecal nicotine administration elicits pressor and tachycardic responses by increasing the spinal sympathetic outflow. As such, in both SHR and WKY rats, the nicotinic agonists would lead to increased blood pressure and heart rate. A blunted baroreceptor reflex, enhanced sympathetic output, and vascular reactivity in SHR may also contribute to exaggerated responses in hypertensive versus normotensive rats. Enhanced pressor responses to central administration of several peptide agonists are also observed in SHR compared with normotensive rats.\textsuperscript{24-26} Also, enhanced depressor responses to amino acids administered in particular brain regions have been documented in SHR relative to WKY rats.\textsuperscript{27,28}

We found a lower density of nicotinic receptors in spinal cord membranes in SHR than in SD or WKY rats. Yamada et al\textsuperscript{12} observed similar decreases in nicotinic receptor number in various brain regions, including the medulla oblongata, of stroke-prone SHR compared with WKY rats. Microinjections of nicotine or acetylcholine in various regions of the medulla, including the dorsal medulla, nucleus of the solitary tract, and area postrema, result in a decrease in blood pressure and heart rate.\textsuperscript{5,11,29} No differences in the responses were observed between SHR and normotensive rats.\textsuperscript{5} However, Tseng et al\textsuperscript{3} demonstrated that microinjections of nicotine in the rostral ventrolateral medulla produce a dose-dependent increase in blood pressure and heart rate in SHR and WKY and SD rats that is blocked by hexamethonium. Moreover, the pressor and tachycardic responses to nicotine were augmented in SHR compared with the normotensive rats. Thus, similar to our observations in spinal cord, an augmented pressor and heart rate response to nicotinic receptor stimulation in the presence of a decreased number of nicotinic receptors could be observed in a different region of the rat central nervous system. The fact that the rostral ventrolateral medulla is sensitive to nicotinic agonists that elicit cardiovascular responses is noteworthy because this region of the brain has been implicated in the tonic and reflex regulation of blood pressure.\textsuperscript{30,31} Moreover, direct innervation from this brain region to the intermediolateral region in the spinal cord has been documented.\textsuperscript{30,32} Thus, it appears that the two separate sites in the brain and spinal cord involved in the neuronal circuitry of regulating tonic and reflex cardiovascular responses are sensitive to nicotine. Moreover, nicotinic receptor stimulation in the two sites elicits similar augmented cardiovascular responses in SHR.

Increased cholinergic activity in various brain regions has been demonstrated in SHR.\textsuperscript{33,34} Moreover, inhibition of the cholinergic system by intracerebroventricular hemicholinium-3 treatment led to a fall in blood pressure in hypertensive rats.\textsuperscript{17} Thus, the lower nicotinic receptor numbers in the brain regions of hypertensive versus normotensive rats could be explained by a feedback mechanism whereby SHR compensate for the increased cholinergic sensitivity in the central nervous system by downregulating cholinergic receptors during the onset of hypertension.

Compared with higher central nervous system centers, little information is available for the cholinergic activity in receptors in spinal cord of SHR. The densities of the nicotinic receptors in the dorsal lumbarosacral, ventral lumbarosacral, and intermediolateral regions, although lower in SHR\textsubscript{LJ}, have a regional distribution similar to that in WKY\textsubscript{LJ} rats. Nicotinic receptor number is highest in the dorsal lumbarosacral region, followed by the intermediolateral and ventral lumbarosacral sections of the spinal cord in both rat strains (Table 3). Moreover, we have shown previously that the intermediolateral region is most sensitive to intrathecal nicotinic agonists in eliciting the pressor response, whereas the entire length of the thoracolumbar spinal column mediates the irritation response to intrathecal nicotinic agonists.\textsuperscript{14} The fact that the SHR\textsubscript{LJ} is the strain most sensitive to the nicotinic agonist–elicited irritation response suggests an enhanced amplification process of the postcoupling events in the dorsal lumbar spinal cord to increase sympathetic activity in the SHR\textsubscript{LJ}. It will be of interest to ascertain whether the decreased receptor number precedes the hypertension or is a feedback manifestation of the apparent enhanced amplification of spinal cholinergic stimulation.

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References


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