Central Modulation of Baroreceptor Reflex Response to Phenylephrine by Dihydropyridines in Rats

PATRICK LACOLLEY, STÉPHANE LAURENT, DANITA TSOUCARIS-KUPFER, MONIQUE LEGRAND, ANNE MARIE BRISAC, AND HENRI SCHMITT

SUMMARY  The effects of two dihydropyridine derivatives, the calcium channel agonist BAY k 8644 or the antagonist PN 200-110, on the central nervous components of the baroreceptor reflex control of heart rate during activation of baroreceptors by phenylephrine (2 µg i.v.) were studied in pentobarbital-anesthetized normotensive (Wistar) rats and spontaneously hypertensive rats (SHR). To rule out an effect on peripheral vessels or on the sinoauricular node (or on both), BAY k 8644 and PN 200-110 were administered intracerebroventricularly (i.c.v.) at doses that did not change blood pressure. Baroreceptor reflex sensitivity was calculated as the slope of the relationship between systolic arterial pressure and heart period. Baroreceptor reflex sensitivity increased with time following the onset of anesthesia. In SHR, injection of BAY k 8644 (3 µg/kg i.c.v.) suppressed the time-dependent increase in baroreceptor reflex sensitivity. The inhibitory effect of BAY k 8644 (3 µg/kg i.c.v.) on the time-dependent increase in baroreceptor reflex sensitivity was suppressed by pretreatment with PN 200-110 (0.6 µg/kg i.c.v.) but not with the solvent, indicating that the central effect of BAY k 8644 occurred at the level of specific dihydropyridine binding sites. In addition, the inhibitory effect of BAY k 8644 (3 µg/kg i.c.v.) on the time-dependent increase in baroreceptor reflex sensitivity was suppressed by pretreatment with the muscarinic antagonist atropine methylnitrate (80 µg/kg i.c.v.) but not with the solvent. In normotensive rats, the time-dependent increase in baroreceptor reflex sensitivity was not significantly altered by BAY k 8644 (3 µg/kg i.c.v.). These results indicate that dihydropyridines may centrally modulate baroreceptor reflex sensitivity in SHR and suggest that the inhibitory effect of the calcium agonist BAY k 8644 on baroreceptor reflex sensitivity may have been mediated by an enhanced release of acetylcholine. (Hypertension 12: 279–286, 1988)

KEY WORDS  • arterial baroreceptor reflexes • dihydropyridines • spontaneously hypertensive rats • cholinergic mechanisms

THE heart rate (HR) response to the systemic administration of calcium channel blockers seems to vary depending on the specific calcium channel blocker selected. Some of these differences may be due to a direct effect of the calcium antagonist on the baroreceptor reflex.1 However, the sites of action for these drugs in the baroreceptor reflex are not well understood.

In most of the earlier studies, baroreceptor reflex function was assessed upon intravenous infusion of the calcium channel antagonist.2-6 Thus, the site of action of the drug could have been anywhere in the baroreceptor reflex pathway, since calcium channel blockers may have modulated the afferent or the efferent part of the pathway (or both) by relaxing the vascular smooth muscle or they may have modulated the central nervous component of the pathway after crossing the blood-brain barrier. The results of the few studies that have attempted to assess the direct effects of calcium channel blockers on the baroreceptors, using carotid sinus or aortic arch preparations,7-8 are somewhat conflicting. Blockade of voltage-dependent calcium channels at therapeutic concentrations did not seem to alter baroreceptor transduction in the work of Kunze et
al.,8 while Heesch et al.7 reported that nifedipine enhanced multifunction baroreceptor activity. Recently, Higuchi et al.9 suggested that a central nervous component of the baroreceptor reflex pathway was sensitive to the dihydropyridine-derived calcium channel antagonists. However, no study of the central effects of dihydropyridines on baroreceptor reflex function has been done.

There are several arguments for the hypothesis that baroreceptor reflex function is modulated centrally by dihydropyridines. Dihydropyridine receptor sites have been found in the brain.10-12 Recent findings support a physiological role for these sites. The dihydropyridine derivatives, either calcium channel agonists or antagonists, have been shown to modulate the stimulated release from brain tissue of neurotransmitters involved in the central control of blood pressure and HR: acetylcholine,13,14 dopamine,13,15 serotonin,16 and norepinephrine.17 Moreover, the effects of dihydropyridines on the release of neurotransmitters were correlated with changes in the net voltage-dependent entry of calcium into synaptosomes.15,17 These data suggest that calcium channel modulators may influence the autonomic nervous system at the central level.

Higuchi et al.9,18 reported an excitatory effect on the nucleus tractus solitarii when nifedipine was administered into the cisterna magna or when the local availability of calcium was reduced, resulting in hypotension and bradycardia in conscious rats. We have previously shown19 that the dihydropyridine derivatives BAY k 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-[2-trifluoromethylphenyl]-pyridine-5-carboxylate), a calcium channel agonist,20 and nifedipine, a calcium channel antagonist, increased and decreased mean arterial pressure (MAP) respectively, when intracerebroventricularly (i.c.v.) injected in anesthetized spontaneously hypertensive rats (SHR) by a mechanism of central origin. No tachycardia accompanied the hypotensive response to i.c.v. administered nifedipine, in contrast to that reported after oral or intravenous administration.21 These findings suggest that central dihydropyridine receptor sites may play a role in the control of blood pressure and baroreceptor reflex function.

In light of this evidence, the present study was designed to examine the effects of the dihydropyridine derivatives PN 200-110 (isopropyl 4-[2,1,3-benzoxadiazol-4-yl]-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridine carboxylate), a calcium channel antagonist,22 or BAY k 8644, a calcium channel agonist,20 on the central nervous component of the baroreceptor reflex control of HR during activation of baroreceptors by phenylephrine. To rule out an effect on peripheral vessels or on the sinoaauricular node (or on both) and to allow conclusions about a central effect, dihydropyridines were administered i.c.v. at doses that did not change MAP. These experiments were performed in anesthetized SHR, in which significant changes in blood pressure were observed in previous experiments after i.c.v. or intracisternal administration of nifedipine or BAY k 8644 as compared with responses in normotensive rats.19 Since the pressor effect of i.c.v. administered BAY k 8644 was reported to involve a central increase in muscarinic cholinergic activity in anesthetized SHR,14 we tested the hypothesis that the BAY k 8644-induced change in baroreceptor reflex sensitivity (BRS) was mediated by a change in central cholinergic activity, using central administration of the muscarinic receptor antagonist atropine methlylnitrate (AMN).

Materials and Methods

Male, 14-week-old normotensive Wistar rats and SHR (Okamoto strain, Ifla-Credo, Fresnes, France) weighing 300 to 350 g were anesthetized with pentobarbital (50 mg/kg i.p.). The trachea was cannulated, and the animals were artificially ventilated by means of a Palmer "Iideal" 10 pump (London, England). Instantaneous pressure and MAP were recorded continuously from a femoral artery by means of a Statham P23Db pressure transducer (Oxnard, CA, USA) on two channels of a Gould-Brush 3302 recorder (Cleveland, OH, USA). HR was continuously recorded from the electrocardiographic signal. A femoral vein was cannulated with a polyethylene catheter for the administration of phenylephrine, propranolol, or AMN.

Central administrations of drugs were performed as previously described.19 Briefly, i.c.v. injections were performed through a cannula inserted into one of the lateral cerebral ventricles through a trephine hole drilled 2 mm lateral and 2 mm posterior to the bregma, at a depth of 3.5 to 4 mm from the top of the skull. All drugs were administered in a 1.5-μl volume for 1 minute. Arterial pressure and HR were recorded for 65 minutes following central administration. In preliminary experiments, increasing doses of PN 200-110 (0.2, 0.6, 2, 10 μg/kg) and BAY k 8644 (1, 3, 10, 30 μg/kg) were administered i.c.v. to SHR to determine the threshold dose for a significant change in MAP. Only one dose of PN 200-110 or BAY k 8644 was administered in each rat.

BRS was determined using the method of Smyth et al.23 with minor modifications. Intravenous injections of phenylephrine (2 μg) were used to induce systolic blood pressure (SBP) changes larger than 20 mm Hg. For each injection, the linear relationship between the drug-induced changes in SBP and heart period (msec) was computed. Since a latency exists in the heart period response that is close to 1 second in humans as well as in rats,24-26 we calculated in preliminary experiments the relationship between SBP and heart period with different shifts (+4 to +10 beats), as indicated by Struyker Boudier et al.24 The highest correlation coefficients and slopes were obtained with a shift of +9 beats, which was used for all calculations. Under these conditions, the slope of the relationship was used as an index of BRS and expressed in milliseconds per
millimeter of mercury (Figure 1). We discarded the few animals in which the correlation coefficient of the relationship between SBP and heart period, determined from at least 10 pairs of values (usually 12–20), was not significant (p > 0.05).

To evaluate the disappearance of the inhibitory effect of pentobarbital anesthesia on BRS, BRS was assessed 30, 40, 55, and 65 minutes after the onset of anesthesia in preliminary experiments. To take into account the time dependency of BRS (Figures 1 and 2) when comparing different i.c.v. treated groups, the timing for i.c.v. injections of ethanol, PN 200-110, or BAY k 8644 and i.v. injections of phenylephrine was the same for each type of experiment, as shown in Figure 3. Basal values of MAP, HR, and BRS were determined 30 (MAP, HR) and 40 minutes (BRS) after the onset of anesthesia. BRS was reassessed 20 minutes after i.c.v. injection of drugs (i.e., at the 65th minute). An experiment was performed in normotensive rats to compare the time dependency of BRS in the absence or presence of BAY k 8644 i.c.v. administered at the 45th minute after the onset of anesthesia.

Three experiments were performed in SHR. Experiment 1 was performed to assess the time dependency of BRS during the 40th to 65th minute and its modifications by i.c.v. injection of ethanol (solvent) or subthreshold doses of PN 200-110 (0.6 µg/kg) or BAY k 8644 (3 µg/kg) at the 45th minute. Experiments 2 and 3 were performed to study the antagonism of the BAY k 8644 (3 µg/kg i.c.v.)-induced decrease in BRS by pretreatment with the calcium channel antagonist PN 200-110 (0.6 µg/kg i.c.v.) or the muscarinic receptor antagonist AMN (80 µg/kg i.c.v.).

PN 200-110 was kindly provided by Dr. J.F. Le Bigot, Sandoz, Rueil-Malmaison, France. BAY k 8644 was kindly provided by Dr. B. Garthoff, Bayer A.G., Wuppertal, Federal Republic of Germany. Phenylephrine, propranolol, and AMN were pur-
chased from Sigma Chemical (St. Louis, MO, USA). Solvent for i.c.v. injections of PN 200-110 and BAY k 8644 was 95% ethanol (vol/vol), which was also used alone as the control solution. Solvent for i.c.v. injections of AMN and i.v. injection of phenylephrine was saline.

Statistical analysis was performed by means of a one-way analysis of variance (ANOVA) followed by a Newman-Keuls test for multiple comparisons, when baseline values of BRS, MAP, and HR were compared, and by a repeated-measure ANOVA followed by a Newman-Keuls test, when comparing the time dependency of BRS, MAP, and HR under i.c.v. pretreatment in different groups. A Student’s t test for paired data was used to compare BRS before and after i.c.v. injection in each group. Values are expressed as means ± SEM. A probability value of less than 0.05 was considered statistically significant.

Results

BRS was significantly lower in SHR than in normotensive Wistar rats at the 40th minute following the onset of anesthesia (0.09 ± 0.03 vs 0.33 ± 0.08 msec/mm Hg; p < 0.02). In both strains, BRS increased with time (see Figure 2). In SHR as well as in normotensive rats, BRS was significantly higher at the 65th minute (p < 0.05) than at the 40th minute (SHR, 0.39 ± 0.08 vs 0.09 ± 0.03 msec/mm Hg; n = 6, p < 0.01; normotensive rats, 0.62 ± 0.10 vs 0.33 ± 0.08 msec/mm Hg; n = 5, p < 0.01).

In SHR, i.v. administration of AMN (1 mg/kg), a muscarinic antagonist that does not readily cross the blood-brain barrier, suppressed the i.v. phenylephrine-induced bradycardia. By contrast, i.v. administration of the β-adrenergic blocker propranolol (1 mg/kg) did not change the phenylephrine-induced bradycardia. Propranolol (1 mg/kg, i.v.) did not change basal BRS and its time-dependent increase (from 0.17 ± 0.05 to 0.33 ± 0.08 msec/mm Hg) as compared with values in control conditions.

Injections of PN 200-110 or BAY k 8644 into the lateral cerebral ventricle 45 minutes after the onset of anesthesia induced a long-lasting (>30 minutes) dose-dependent decrease and increase in blood pressure, respectively, with threshold doses of 2 and 10 μg/kg. The maximum changes in MAP after i.c.v. administration of PN 200-110 (0.2, 0.6, 2, and 6 μg/kg) were, respectively, +6 ± 3 (n = 5, NS), −7 ± 4 (n = 7, NS), −28 ± 6 (n = 6, p < 0.05), and −56 ± 8 mm Hg (n = 5, p < 0.01). The maximum changes in MAP after i.c.v. administration of BAY k 8644 (1, 3, 10, and 30 μg/kg) were, respectively, +2 ± 2 (n = 5, NS), +3 ± 2 (n = 5, NS), +16 ± 5 (n = 8, p < 0.05), and +25 ± 8 mm Hg (n = 5, p < 0.05). Therefore, a 0.6 μg/kg dose of PN 200-110 and a 3 μg/kg dose of BAY k 8644 were considered subthreshold, as concerns the changes in MAP, and were used in the subsequent experiments.

In normotensive rats, i.c.v. injection of BAY k 8644 (3 μg/kg) did not change significantly the time-dependent increase in BRS observed from the 40th to the 65th minute (from 0.30 ± 0.09 to 0.64 ± 0.11 msec/mm Hg; n = 6, p < 0.05) as compared with changes seen in the control group (from 0.33 ± 0.08 to 0.62 ± 0.10 msec/mm Hg; p < 0.01, n = 5). During this period, no change in MAP was significant in either group.

The time-dependent increase in BRS during the 40th to 65th minute (from 0.11 ± 0.04 to 0.35 ± 0.09 msec/mm Hg; n = 8, p < 0.01) in SHR receiving an i.c.v. injection of the solvent at the 45th minute was not significantly different from the time-dependent increase in BRS in the control group of SHR receiving no i.c.v. injection (from 0.09 ± 0.03 to 0.39 ± 0.08 msec/mm Hg; n = 6, p < 0.01). Similarly, during Experiment 1, the time-dependent increase in BRS during the 40th to 65th minute (from 0.17 ± 0.04 to 0.61 ± 0.16 msec/mm Hg; n = 9, p < 0.02) in SHR receiving an i.c.v. injection of PN 200-110 (0.6 μg/kg) was not significantly different from the time-dependent increase in BRS in SHR receiving an i.c.v. injection of the solvent (by ANOVA; Figure 4). By contrast, i.c.v. administration of BAY k 8644 (3 μg/kg) suppressed the time-dependent increase in BRS during the 40th to 65th minute (from 0.11 ± 0.03 to 0.14 ± 0.05 msec/mm Hg; n = 8; see Figure 4). The difference between 1) the ethanol-induced or PN 200-110-induced change in BRS and 2) the BAY k 8644-induced change in BRS was significant (p < 0.05 and p < 0.01, respectively, by ANOVA). Basal values of MAP, HR, and BRS were not significantly different among the three groups (Table 1). MAP and HR, recorded immediately before each of the two i.v. phenylephrine injections (i.e., at the 40th

![Figure 4. Experiment 1: Baroreceptor reflex sensitivity (BRS) before (C) and after (C) i.c.v. administration of PN 200-110 (PN; 0.6 μg/kg; n = 9), ethanol (1.5 μl; n = 8), or BAY k 8644 (BAY, 3 μg/kg; n = 8) in SHR. Open and hatched columns refer to BRS assessed at the 40th (before i.c.v. injection of drugs or solvent) and the 65th minutes (after i.c.v.) following the onset of anesthesia, respectively, as shown in Figure 2. As indicated by the star, BAY k 8644 significantly suppressed the time-dependent increase in BRS (p < 0.01, by ANOVA) while PN 200-110 or the solvent did not. Values are means ± SEM.](http://hyper.ahajournals.org/content/12/3/282)
and the 65th minutes after anesthesia), were not significantly different after i.c.v. injection of drugs or solvent, as compared with values before i.c.v. injection, in the three groups (Table 2). The pressor effect of i.v. injected phenylephrine (2 μg) was unchanged after i.c.v. administration of PN 200-110 (0.6 μg/kg; before PN 200-110 the change was +78 ± 3 mm Hg; after PN 200-110 the change was +84 ± 3 mm Hg) or BAY k 8644 (3 μg/kg; before BAY k 8644 the change was +68 ± 3 mm Hg). The difference between BAY k 8644–induced change in BRS and PN 200-110 plus BAY k 8644–induced change in BRS was significant (p < 0.02, by ANOVA), as was the difference between ethanol plus BAY k 8644–induced and PN 200-110 plus BAY k 8644–induced change in BRS (p < 0.02). Basal values of MAP, HR, and BRS were not significantly different among the three groups (see Table 1). MAP and HR, recorded immediately before each of the two i.v. phenylephrine injections (i.e., at the 40th and the 65th minutes after onset of anesthesia), were not significantly different after i.c.v. injection, as compared with values before i.c.v. injection, in the three groups (see Table 2).

In Experiment 3, i.c.v. administration of AMN (80 μg/kg) did not change the time-dependent increase in BRS, as compared with that for the solvent (i.c.v. administration of AMN: from 0.11 ± 0.06 to 0.36 ± 0.09 msec/mm Hg; n = 5, p < 0.05; i.c.v. administration of ethanol: from 0.11 ± 0.04 to 0.35 ± 0.09 msec/mm Hg; n = 8, p < 0.01; Figure 6).

Table 1. Basal Values of MAP, Heart Rate, and Baroreceptor Reflex Sensitivity in Anesthetized SHR

<table>
<thead>
<tr>
<th>Experiment</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>BRS (msec/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (n = 8)</td>
<td>153 ± 9</td>
<td>373 ± 13</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>PN (n = 9)</td>
<td>145 ± 5</td>
<td>368 ± 7</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>BAY (n = 8)</td>
<td>141 ± 3</td>
<td>351 ± 8</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Ethanol + BAY (n = 6)</td>
<td>163 ± 8</td>
<td>320 ± 20</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>PN + BAY (n = 7)</td>
<td>158 ± 8</td>
<td>339 ± 9</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Ethanol (n = 6)</td>
<td>148 ± 4</td>
<td>358 ± 9</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>AMN (n = 5)</td>
<td>165 ± 12</td>
<td>357 ± 12</td>
<td>0.11 ± 0.06</td>
</tr>
<tr>
<td>AMN + BAY (n = 7)</td>
<td>162 ± 7</td>
<td>344 ± 11</td>
<td>0.14 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SEM before i.c.v. injection of the calcium channel agonist BAY k 8644, 3 μg/kg, the calcium channel antagonist PN 200-110, 0.6 μg/kg, or the muscarinic antagonist atropine methyl nitrate (AMN), 80 μg/kg. HR = heart rate; BRS = baroreceptor reflex sensitivity; PN = PN 200-110; BAY = BAY k 8644.

One-way analysis of variance was done for each experiment. No significant difference was observed. All drugs were administered into a 1.5 μl volume of ethanol.

Table 2. Effect of i.c.v. Injection of the Calcium Channel Agonist BAY k 8644, the Calcium Channel Antagonist PN 200-110, or the Muscarinic Antagonist Atropine Methyl nitrate on MAP and Heart Rate in Anesthetized SHR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before injection</th>
<th>After injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>HR (beats/min)</td>
</tr>
<tr>
<td>Ethanol (n = 8)</td>
<td>162 ± 8</td>
<td>378 ± 17</td>
</tr>
<tr>
<td>PN (n = 9)</td>
<td>149 ± 6</td>
<td>360 ± 8</td>
</tr>
<tr>
<td>BAY (n = 8)</td>
<td>150 ± 3</td>
<td>349 ± 11</td>
</tr>
<tr>
<td>Ethanol + BAY (n = 6)</td>
<td>175 ± 8</td>
<td>359 ± 26</td>
</tr>
<tr>
<td>PN + BAY (n = 7)</td>
<td>167 ± 12</td>
<td>341 ± 12</td>
</tr>
<tr>
<td>Ethanol (n = 6)</td>
<td>155 ± 4</td>
<td>352 ± 10</td>
</tr>
<tr>
<td>AMN (n = 5)</td>
<td>172 ± 12</td>
<td>360 ± 18</td>
</tr>
<tr>
<td>AMN + BAY (n = 7)</td>
<td>163 ± 8</td>
<td>359 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SEM. HR = heart rate; PN = PN 200-110; BAY = BAY k 8644; AMN = atropine methyl nitrate.

A repeated-measure analysis of variance was done for each experiment. No significant difference was observed.

Doses as in Table 1.
However, i.c.v. pretreatment with AMN (80 μg/kg) antagonized the inhibitory effect of BAY k 8644 on the time-dependent increase in BRS (without AMN: from 0.11 ± 0.04 to 0.13 ± 0.03 msec/mm Hg; n = 6; with AMN: from 0.14 ± 0.04 to 0.42 ± 0.10 msec/mm Hg; n = 7, p < 0.05). The difference between the BAY k 8644–induced change in BRS and the AMN plus BAY–induced change in BRS was significant (p < 0.05, by ANOVA). Basal values of MAP, HR, and BRS were not significantly different among the three groups (see Table 1). MAP and HR, recorded immediately before each of the two phenylephrine injections, were not significantly different after i.c.v. injection, as compared with values before i.c.v. injection, in the three groups (see Table 2).

**Discussion**

Our results indicate that a calcium channel agonist and a calcium channel antagonist may modulate the baroreceptor reflex response to phenylephrine in pentobarbital-anesthetized SHR. BRS was evaluated from the slope of the relationship between systolic arterial pressure and heart period. In the present study, we focused on the changes in the time course of BRS in response to i.c.v. administration of dihydropyridines.

In anesthetized rats, arterial blood pressure variability was obviously reduced as compared with that in conscious rats. However, the inhibitory effects of pentobarbital anesthesia on BRS had to be taken into account in the interpretation of the results. The time-dependent increase in BRS was likely due to the time-dependent decrease in the inhibitory effect of anesthesia. Forty minutes after pentobarbital injection, BRS was 0.33 ± 0.08 msec/mm Hg in normotensive rats and averaged 0.11 ± 0.01 msec/mm Hg in SHR. Sixty-five minutes after the onset of anesthesia, BRS was 0.62 ± 0.10 and 0.39 ± 0.08 msec/mm Hg in normotensive rats and in SHR, respectively. These values are close to those reported by others in conscious animals. The difference in BRS between the 65th and the 40th minutes is consistent with the twofold to threefold decrease in BRS caused by pentobarbital anesthesia in rats.

In SHR, BAY k 8644, administered i.c.v. at the 45th minute after onset of anesthesia, suppressed the time-dependent increase in BRS, since BRS determined at the 65th minute was not significantly different from BRS determined at the 40th minute. This effect was likely due to a specific interaction of BAY k 8644 with central dihydropyridine receptors, since 1) it did not occur with the vehicle injected in the same volume as drug solution, 2) the i.c.v. injection of the structurally close compound PN 200-110, in a solution of equal volume and nearly identical osmolarity, induced no change in the time-dependent increase in BRS, and 3) the inhibitory effect of i.c.v. administered BAY k 8644 on the time-dependent increase in BRS was antagonized by a previous i.c.v. injection of PN 200-110 but not by the solvent. These results suggest the involvement of specific dihydropyridine binding sites in the central modulation of the baroreceptor reflex.
response to phenylephrine, in agreement with previous findings on the central modulation of blood pressure by dihydropyridines.9-14, 19

The inhibitory effect of BAY k 8644 on BRS probably was not due to interaction with anesthetics. Calcium channel inhibitors have been shown to potentiate the anesthetic effect of pentobarbital.29, 30 In our model, in which pentobarbital decreased BRS, the calcium channel inhibitor PN 200-110 should have decreased BRS; however, BRS was not significantly different after PN 200-110, as compared with control conditions. In addition, BAY k 8644 should have increased BRS by suppressing the inhibitory effect of pentobarbital on BRS. In our model, BAY k 8644 had the opposite effect. Therefore, an interaction with pentobarbital was unlikely.

The inhibitory effect of i.c.v. BAY k 8644 administration on the time-dependent increase in BRS likely occurred at the level of the central integration of the baroreceptor reflex. A possible effect of BAY k 8644 on baroreceptors on the afferent side and on the vagal synapse of the sinoauricular node was ruled out since dihydropyridines were i.c.v. administered at doses that did not change MAP. However, the afferent axons and their synapses in the nucleus tractus solitarii as well as the vagal preganglionic neurons were exposed to the drugs since they are within the central nervous system. During each experiment, the level of MAP was carefully controlled and the few experiments in which MAP changed after i.c.v. injection were discarded. Therefore, i.c.v. administration of BAY k 8644 and PN 200-110 cannot have changed baroreceptor activation through changes in distending pressure or vascular distensibility (or both) at the level of the baroreceptors or changed baroreceptor output through modulation of the vagal synapse of the sinoauricular node. Thus, their effects on the baroreceptor reflex response were likely of central origin.

Although one may speculate on the mode of action, there is no way to identify the location of any observed effect after i.c.v. injection. The calcium channel agonist and antagonist are likely to act at almost every synapse and on every neuron in the central nervous system. The overall effect will depend on the specific cell activities during the experimental procedures and on the voltage dependence characteristic of the dihydropyridine being used. However, the purpose of this work was limited to the demonstration that calcium channel agonists and antagonists can modulate the central nervous component of the baroreceptor reflex control of HR. Since dihydropyridine derivatives may easily pass the blood-brain barrier after peripheral administration, a central effect of these drugs on the baroreceptor reflex control of HR is important to consider in their overall effect on blood pressure and HR.

With the rats under pentobarbital anesthesia, the phenylephrine-induced bradycardia was mainly due to an increase in vagal tone, since i.v. AMN, a muscarinic receptor antagonist that does not readily cross the blood-brain barrier, suppressed the i.v. phenylephrine-induced bradycardia. In addition, i.v. administration of the β-adrenergic blocker propranolol did not change basal BRS and its time-dependent increase as compared with values in control conditions. However, the smaller phenylephrine-induced bradycardia observed after i.c.v. BAY k 8644 injection, as compared with values in control conditions, may have been due to a smaller increase in vagal tone or to a tonic increase in sympathetic tone unrelated to baroreceptor reflex activation, which may have buffered the baroreceptor reflex-mediated vagal stimulation. The second hypothesis is in agreement with previous reports14, 19 showing, in SHR, an increase in sympathetic tone leading to an increase in blood pressure in response to i.c.v. administration of BAY k 8644. Since BAY k 8644 was, in the present study, i.c.v. administered at a dose that did not change blood pressure, we have to assume that this dosage was high enough to modulate the central integration of the baroreceptor reflex and low enough to induce no change in basal blood pressure.

Central cholinergic stimulation in several species, including rat, generally evoked a hypertensive response mediated by a central increase in sympathetic tone.31 Brisac et al.14 have reported that the pressor effect of i.c.v. administration of BAY k 8644 in SHR involved an increased muscarinic cholinergic activity. In the present study, i.c.v. administered AMN did not change the time-dependent increase in BRS. However, when AMN was i.c.v. injected before BAY k 8644, it antagonized the inhibitory effect of BAY k 8644 on the time-dependent increase in BRS. These results suggest that BAY k 8644 and acetylcholine may have been acting on the same pathway. In addition, the inhibitory effect of BAY k 8644 on the time-dependent increase in BRS may have been mediated by a central release of acetylcholine, the cholinergic-mediated increase in sympathetic tone buffering the baroreceptor reflex-mediated vagal stimulation and decreasing BRS.

In normotensive rats, i.c.v. BAY k 8644 administration did not change the time-dependent increase in BRS. As previously shown,14, 19 the cardiovascular effects of BAY k 8644 and calcium channel inhibitors were more marked in SHR than in normotensive rats. Ishii et al.32 reported a 57% increase in the maximum number of binding sites for [3H]nitrendipine (a dihydropyridine molecule) in brain membranes of SHR as compared with those in normotensive Wistar-Kyoto rats. The increased number of dihydropyridine binding sites in SHR was not secondary to the high blood pressure but could be a primary abnormality in SHR.32 Whether this increase in the maximum number of binding sites reflects an increase in functional calcium channels and explains the increased responsiveness of SHR to the centrally administered calcium channel modulators.
remains to be determined. Brisac et al.\textsuperscript{14} showed that BAY k 8644 did not change the K\textsuperscript+ -evoked release of acetylcholine in hippocampal slices from normotensive rats, while BAY k 8644 increased it in SHR, and they suggested that the increased cholinergic activity in SHR could be related, at least in part, to an increased number of dihydropyridine binding sites. Under these conditions, the stimulation of these sites by a specific and still unknown neuromediator could lead to an enhanced release of acetylcholine and an increase in sympathetic tone and thus could participate in the genesis or the maintenance (or both) of decreased BRS in SHR. Further studies are required to assess the relative role of the cholinergic pathway involved in the effects of BAY k 8644 on the BRS as compared with pathways involving other neurotransmitters.

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P Lacolley, S Laurent, D Tsoucaris-Kupfer, M Legrand, A M Brisac and H Schmitt

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