Role of Nisoldipine on Blood Pressure, Cardiac Hypertrophy, and Atrial Natriuretic Peptides in Spontaneously Hypertensive Rats

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SUMMARY The effect of long-term treatment with the calcium antagonist nisoldipine on development of hypertension, cardiac hypertrophy, and plasma levels of atrial natriuretic peptides (ANP) was determined in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) of the same age. Measurement of immunoreactive ANP in plasma provided a sensitive marker for the severity of hypertension and the associated cardiac overload. Long-term treatment with nisoldipine prevented the development of hypertension, the associated heart failure, and the increase of plasma levels of ANP in SHR but had no effect on systolic blood pressure, heart weight, and plasma levels of ANP in WKY. In addition, nisoldipine had a therapeutic effect in old SHR with manifest cardiac failure in end-stage hypertension, as evidenced not only by the reduction of blood pressure but also by the reduction of cardiac hypertrophy, of elevated immunoreactive ANP in plasma, and of increased plasma renin activity. (Hypertension 10: 303-307, 1987)

KEY WORDS • atrial natriuretic peptide • hypertension • Ca\(^{2+}\) antagonism • heart failure • spontaneously hypertensive rat • radioimmunoassay • monoclonal antibody • nisoldipine

CARDIAC atria are thought to participate in the regulation of fluid volume, electrolyte homeostasis, and blood pressure through the release of peptides that cause diuresis, natriuresis, and reduction in blood pressure (for a review see References 1 and 2). These atrial natriuretic peptides (ANP) are released into the circulation in response to atrial stretching.\(^3,4\) Since several lines of evidence in experimental models of hypertension suggest a close link between sodium and water regulation and development of hypertension it is of great importance to ascertain whether ANP is involved in the pathogenesis of this disorder.

Plasma levels of ANP in spontaneously hypertensive rats (SHR) have been shown to increase with the development of hypertension in comparison to levels in age-matched Wistar-Kyoto rats (WKY).\(^5,6\) but little information is available about ANP levels in end-stage hypertension and their modulation by antihypertensive treatment. We therefore investigated the effect of the calcium antagonist nisoldipine on blood pressure, heart weight, and ANP levels in plasma when continuously administered in a preventive regimen from prehypertensive age in SHR. In addition to this preventive experiment, therapeutic treatment with nisoldipine was performed in old SHR with manifest cardiac failure in the end-stage of hypertension. As late-stage hypertension is associated with renal damage in SHR, plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were also determined.

Materials and Methods

In the preventive experiment, 8-week-old male SHR (Moellegaard, Ejby, Denmark) and normotensive WKY (Moellegaard) were treated with 1000 ppm nisoldipine mixed into the commercial diet (ssniff R; ssniff Versuchstierdiäten GmbH, Soest, Federal Republic of Germany), equivalent to a daily dose of 50 to 100 mg/kg body weight according to the mean daily food consumption at different ages. This method of drug administration using this dose ensured a continuous lowering of blood pressure in rats throughout the...
day. Untreated SHR and WKY of the same age served as controls.

In the therapeutic experiment, two groups of 8-week-old SHR were treated with 1000 ppm of nisoldipine in the solid feed and two groups of age-matched SHR served as controls. After 61 weeks of observation the treatment in one of the nisoldipine-treated groups was finished while the same therapeutic treatment was applied in one of the untreated groups. In the other two groups the regimen was not changed. All groups were then observed for another 10 weeks.

In the preventive and therapeutic experiment systolic blood pressure was measured by the tail-cuff method in conscious animals, prewarmed in thermostatic cages at 37°C every 2 weeks. 7

At the end of the experiment, all animals were weighed and killed by decapitation at 0900 to 1100. After thoracotomy, the hearts were removed and the ventricles isolated by cutting off the atria, pulmonary arteries, and aortas. The ventricles were opened, washed, dried with filter paper, and weighed. Blood samples were collected after decapitation into pre-chilled tubes.

Immunoreactive ANP in Plasma

The radioimmunological determination of immunoreactive ANP (irANP) using a monoclonal antibody directed against ANP and the preparation of plasma samples was performed as previously described. 8 Briefly, irANP was separated from plasma by extraction on Sep-Pak C18 cartridges (Waters Associates, Milford, MA, USA), lyophilized, and assayed. For the radioimmunological procedure, we first incubated ascitic fluid, containing the monoclonal antibody (final dilution, 1:1,600,000) in the presence of either atriopeptin II or sample for 4 hours and then added 125I-atrionpeptin III (prepared by the chloramine-T method) for an additional 16-hours incubation. Free peptide was separated from antibody-bound peptide by charcoal separation. The activity of the supernatant was determined in a gamma counter. The monoclonal antibody reacts equally well with atriopeptin I, atriopeptin II, and atriopeptin III, while there is no cross-reactivity to ANP fragment 13–28. The sensitivity of this assay is 10 pg/ml.

Plasma Renin Activity and Aldosterone Concentration

PRA was determined by incubation of rat EDTA plasma with phenylmethylsulfonyl fluoride by measuring the accumulated angiotensin I by a commercially available radioimmunoassay kit (Sorin Biomedica, Saluggia, Italy). Aldosterone concentration in rat plasma was measured by a commercially available radioimmunoassay kit (Sorin Biomedica).

Statistical Tests

Statistical analysis was done using Student’s t test for unpaired data. Standard methods were used to calculate the correlation coefficient (r). All values in tables and figures are means ± SEM.

Results

Preventive Experiment

From the age of 8 weeks, the control SHR progressively manifested sustained hypertension. After 10 weeks of observation (18 weeks of age), systolic blood pressure exceeded 220 mm Hg and then slowly increased during the following 40 weeks to about 250 mm Hg (Figure 1). After 50 weeks systolic blood pressure dropped to about 200 mm Hg (“decapitated hypertension”). In the last week of observation three of 10 animals died. A 60-week nisoldipine treatment of SHR from the age of 8 weeks on effectively prevented the development of hypertension throughout the whole treatment period (see Figure 1). In contrast to the control SHR, the blood pressure of the treated SHR was nearly the same as that of treated and untreated WKY of the same age. The final values of systolic blood pressure of all groups after 60 weeks of observation are shown in Table 1.

Plasma irANP was dramatically increased with respect to values in the control WKY after 60 weeks of observation (68 weeks of age). Long-term treatment with nisoldipine for 60 weeks prevented not only the development of hypertension but also the increase of plasma irANP and attenuated cardiac hypertrophy in SHR. In WKY, however, long-term treatment with nisoldipine had no effect on systolic blood pressure, plasma irANP, and heart weight. The development of body weight was nearly the same in all groups (for final body weight, see Table 1). PRA and PAC were lower in the nisoldipine-treated SHR than in untreated SHR (see Table 1).

Therapeutic Experiment

The therapeutic experiment was performed in parallel for 61 weeks with two control groups and two nisoldipine-treated groups. After 61 weeks of observation the treatment in one of the nisoldipine-treated

FIGURE 1. Preventive experiment: development of systolic blood pressure in male SHR. Note the decline of blood pressure in advanced age. Treatment with nisoldipine for 60 weeks prevented the development of hypertension. Shaded areas indicate SEM.
TABLE 1. Preventive Experiment: The Effect of Long-term Treatment (60 weeks) with the Calcium Antagonist Nisoldipine on Systolic Blood Pressure, Plasma irANP, Relative Heart Weight, Body Weight, PRA, and Plasma Aldosterone Concentration in SHR and WKY

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>SHR Controls (n = 7)</th>
<th>SHR Nisoldipine (n = 10)</th>
<th>WKY Controls (n = 8)</th>
<th>WKY Nisoldipine (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>214 ± 7</td>
<td>141 ± 3‡</td>
<td>145 ± 3‡</td>
<td>137 ± 3</td>
</tr>
<tr>
<td>Plasma irANP (pg/ml)</td>
<td>470 ± 38</td>
<td>139 ± 35‡</td>
<td>88 ± 23‡</td>
<td>107 ± 29</td>
</tr>
<tr>
<td>Relative heart weight (mg/100 g body wt)</td>
<td>376 ± 29</td>
<td>313 ± 4*</td>
<td>277 ± 16†</td>
<td>284 ± 16</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>388 ± 8</td>
<td>377 ± 9</td>
<td>380 ± 16</td>
<td>381 ± 20</td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>2.9 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>PAC (pg/ml)</td>
<td>332 ± 26</td>
<td>242 ± 16†</td>
<td>369 ± 30</td>
<td>454 ± 28</td>
</tr>
</tbody>
</table>

Values are means ± SEM. SBP = systolic blood pressure; irANP = immunoreactive ANP; ANG I = angiotensin I; PRA = plasma aldosterone concentration.

* p < 0.025; † p < 0.01, ‡ p < 0.001, compared with values in untreated SHR.

The potent antihypertensive effect of long-term treatment with the 1,4-dihydropyridine calcium antagonist nisoldipine was clearly demonstrated in these experiments. Nisoldipine prevented the spontaneous development of hypertension in young SHR and decreased blood pressure in old SHR with end-stage hypertension. Cardiac hypertrophy was also prevented in the long-term-treated animals and reduced in old SHR after treatment with nisoldipine. On the other hand, systolic blood pressure immediately increased in these SHR when treatment was discontinued after 61 weeks. Our findings are in accordance with previous results showing that the effects of calcium antagonists on blood pressure are much stronger in SHR than in normotensive WKY,9,10 as well as in humans with essential hypertension compared to corresponding normo-

Discussion

The potent antihypertensive effect of long-term treatment with the 1,4-dihydropyridine calcium antagonist nisoldipine was clearly demonstrated in these experiments. Nisoldipine prevented the spontaneous development of hypertension in young SHR and decreased blood pressure in old SHR with end-stage hypertension. Cardiac hypertrophy was also prevented in the long-term-treated animals and reduced in old SHR after treatment with nisoldipine. On the other hand, systolic blood pressure immediately increased in these SHR when treatment was discontinued after 61 weeks. Our findings are in accordance with previous results showing that the effects of calcium antagonists on blood pressure are much stronger in SHR than in normotensive WKY,9,10 as well as in humans with essential hypertension compared to corresponding normo-

Figure 2. Therapeutic experiment: development of systolic blood pressure in nisoldipine-treated and untreated male SHR (2 control groups and 2 nisoldipine-treated groups). After 61 weeks of observation, the treatment in one of the nisoldipine-treated groups was changed by crossover with one of the untreated groups. The final values of systolic blood pressure are listed in Table 2.
tensive controls. In spite of a pronounced reduction of heart weight in SHR, nisoldipine had no effect on heart weight in WKY.

End-stage hypertension in SHR is associated with cardiac failure as can be concluded from the decrease in systolic blood pressure ("decapitated hypertension") despite cardiac hypertrophy in old, untreated SHR. Moreover, it has been shown that the cardiac performance in SHR is already diminished at the age of 6 months, the ejection fraction being reduced below 50%. In parallel, plasma levels of irANP are several-fold higher in 68-week-old SHR than in age-matched WKY. This result is in accordance with the smaller difference in plasma ANP levels reported for younger SHR with respect to age-matched WKY. It is also reported that in rats with chronic heart failure secondary to healed myocardial infarction, chronic stimulation of ANP release is associated with elevated plasma ANP levels and depleted ANP stores in proportion to the severity of heart failure. These results also suggest that plasma ANP levels can be used as an index of cardiac decompensation.

Although the kinetics of ANP degradation were not studied, it can be assumed that the high plasma ANP levels in SHR are due to a stimulation of ANP release by cardiac overload, as an increase in atrial filling pressure is known to be a strong stimulus for ANP release in animals and humans. Obviously, the ANP system responds to the stimulus of cardiac overload in SHR, and the development of hypertension in SHR cannot be due to a total lack of ANP secretion. However, it cannot be excluded that the response of the ANP system to incremental changes in cardiac filling pressure is lower than that in other strains. This question needs further investigation.

Long-term treatment with nisoldipine prevented the development of hypertension and reduced the associated cardiac hypertrophy almost completely. Interestingly, nisoldipine also had a therapeutic effect in old SHR, as evidenced not only by the reduction of blood pressure but also by the reduction of cardiac hypertrophy and of the increased ANP levels in plasma. Thus, the nisoldipine-induced normalization of plasma ANP levels in SHR must be regarded as the consequence of a protective effect against hypertension and cardiac volume overload. In parallel to these results, a symptomatic improvement in patients with heart failure through different therapeutics was also accompanied by a reduction of the excessively high plasma ANP levels.

Moreover, therapeutic treatment in 22-week-old SHR with the calcium antagonist nitrendipine for 11 weeks resulted in a decrease of systolic blood pressure, a regression of cardiac hypertrophy, and a reduction of elevated plasma ANP levels in comparison to values in the untreated group (135 ± 15 [SEM] pg/ml, n = 14, vs 309 ± 38 pg/ml, n = 19). On the other hand, effective control of blood pressure could also be achieved in the same therapeutic regimen with the sodium-retaining vasodilator minoxidil. In contrast to the calcium antagonist, therapeutic treatment with minoxidil in adult SHR resulted in an aggravation of cardiac hypertrophy and an increase in plasma ANP levels (401 ± 18 [SEM] pg/ml, n = 14, vs 309 ± 38 pg/ml, n = 19: Stasch, unpublished results, 1987).

In isolated, perfused rat hearts, BAY k 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-[2-trifluoromethyl-phenyl]-pyridine-5-carboxylate), a voltage-sensitive calcium channel agonist, was found to stimulate the basal secretory rate of ANP. Conversely, an inhibitory effect of calcium ions on ANP release from perfused rat atria also has been reported. However, in vivo a direct inhibitory action on ANP release by the calcium antagonist nisoldipine seems improbable, since the plasma ANP levels were only reduced in SHR and not in WKY after treatment (see Table 1).

Long-term treatment with calcium antagonists such as nifedipine or nitrendipine, leads to a reduction of PRA in old SHR in parallel with a blood pressure reduction. Obviously, high renin levels in the untreated controls were due to renal ischemia-stimulated renin release. Possibly, nisoldipine also has a protective effect against renal ischemia caused by long-standing hypertension or old age (or both). This becomes evident in this long-term experiment by the prevention or reduction of increased PRA and PAC. The differences in PRA between untreated SHR and

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>SHR treated with nisoldipine</th>
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<tbody>
<tr>
<td>after 71 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>Controls (n=5)</td>
<td>Week 62-71 (n=7)</td>
<td>Week 1-61 (n=11)</td>
<td>Week 1-71 (n=10)</td>
</tr>
<tr>
<td></td>
<td>210 ± 10</td>
<td>169 ± 8†</td>
<td>194 ± 4</td>
<td>181 ± 5‡</td>
</tr>
<tr>
<td>Plasma irANP (pg/ml)</td>
<td>403 ± 43</td>
<td>274 ± 35*</td>
<td>240 ± 34</td>
<td>196 ± 21§</td>
</tr>
<tr>
<td>Relative heart weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/100 g body wt)</td>
<td>434 ± 51</td>
<td>347 ± 4*</td>
<td>328 ± 5</td>
<td>324 ± 7‡</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>376 ± 4</td>
<td>365 ± 13</td>
<td>373 ± 14</td>
<td>374 ± 6</td>
</tr>
<tr>
<td>PRA (ng ANG l/ml/hr)</td>
<td>5.8 ± 0.8</td>
<td>4.3 ± 0.7</td>
<td>2.3 ± 0.3</td>
<td>1.7 ± 0.3§</td>
</tr>
<tr>
<td>PAC (pg/ml)</td>
<td>468 ± 13</td>
<td>515 ± 36</td>
<td>261 ± 16</td>
<td>239 ± 21§</td>
</tr>
</tbody>
</table>

Values are means ± SEM. See Table 1 for key to abbreviations.

* p<0.05, † p<0.025, ‡ p<0.01, § p<0.001, compared with values in untreated controls.
WKY are reported to be strain-specific. On the other hand, a current working theory suggests that agents that reduce PRA reduce cardiac hypertrophy, while those that stimulate this system (e.g., the vasodilator minoxidil) aggravate cardiac hypertrophy. It is therefore not surprising that heart weights were reduced in parallel with PRA in the treated SHR, especially considering that salt and water retention did not occur because of the primary natriuretic activity of the calcium antagonists. It is therefore suggested that reduced volume load together with the reduced peripheral resistance are responsible for the prevention of cardiac hypertrophy and cardiac overload.

In conclusion, the increase of plasma ANP levels in hypertension and the prevention or reduction of this effect by antihypertensive treatment with calcium antagonists show that the changes in plasma ANP levels are secondary to the hypertensive disease and the associated cardiac overload. Thus, the increase of plasma ANP levels could even be regarded as a qualitative marker for cardiac volume overload. This hypothesis is supported by the correlation of irANP in plasma with relative heart weight, but not with blood pressure, in the therapeutic experiment.

References
19. Ruskoski N, Toth M, Ganten D, Enrique T, Lang RE. The phosphoryl ester induced atrial natriuretic peptide secretion is stimulated by forskolin and BAY k 8644 and inhibited by 8-bromo-cyclic GMP. Biochem Biophys Res Commun 1986;139:266–274
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