Abated cardiovascular responses to chronic oral lisinopril treatment in conscious elderly rats

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Abated cardiovascular responses to chronic oral lisinopril treatment in conscious elderly rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1408–R1415, 1999.—To determine whether the cardiovascular effects of chronic treatment with lisinopril are age related, we compared baroreflex sensitivity and pressor responsiveness in 4-mo- and 21-mo-old male rats that had been given oral lisinopril daily for 4 wk. Reflex bradycardia elicited by elevating blood pressure with phenylephrine was stronger in 4-mo-old rats than it was in 21-mo-old rats and also stronger in lisinopril-treated rats than it was in untreated rats of the same age. Pressor responses to angiotensin or norepinephrine were recorded after combined cholinergic and β-adrenergic blockade and then analyzed not only as absolute but also as percent increases in mean pressure. Although pressor responses seemed to be slightly reduced by lisinopril when expressed as absolute increases in mean pressure, corresponding percent increases were always larger in 4-mo-old rats than they were in 21-mo-old rats and were clearly enhanced by lisinopril more in younger rats. The stronger overall enhancement of pressor responsiveness and reflex bradycardia in younger rats suggests that the cardiovascular effects of lisinopril diminish with advancing age.

ANGIOTENSIN-CONVERTING ENZYME (ACE) inhibitors, used as antihypertensive drugs, act mainly by preventing angiotensin-induced vasoconstriction but can also lower blood pressure by enhancing baroreflex sensitivity and reducing pressor responsiveness. Early reports on the baroreflex effects of ACE inhibitors were inconsistent because, although baroreflex sensitivity either increased (11, 12) or was unaffected (21, 31) in clinical studies on normotensive or hypertensive patients, it always increased in experimental studies on normotensive rabbits (33) or spontaneously hypertensive rats (SHR) (2, 23, 24). On the other hand, pressor responsiveness to norepinephrine has been invariably reduced by ACE inhibitors. Hence, norepinephrine responses have always been attenuated by various ACE inhibitors in patients who are normotensive (19), hypertensive (15), or awaiting cardiac surgery (26) as well as in anesthetized rats (32) or isolated SHR arteries (30, 34).

In elderly hypertensives, ACE inhibitors lower blood pressure as effectively (9, 25) as the diuretics and calcium channel antagonists currently recommended for antihypertensive treatment in the elderly (20). This effectiveness, despite the low plasma renin levels in elderly hypertensives, could be caused, at least in part, by changes in baroreflex sensitivity or pressor responsiveness. Thus the baroreflex impairment that normally occurs in elderly hypertensives is alleviated by enalapril (29) or lisinopril (12). Whether ACE inhibitors also act by altering pressor responsiveness in the elderly remains uncertain.

Rat models are suitable for determining the age-related baroreflex effects of ACE inhibitors because baroreflex function deteriorates with age in rats (7) as it does in humans (16). However, although research on aging has often been done on Fischer 344 rats, the Fischer 344 strain may be inappropriate for studying age-related cardiovascular changes because the rats do not readily develop atherosclerosis or hypertension (4). Responses to ACE inhibitors may also differ between rat strains, such as when 2-wk intracerebroventricular infusions of enalapril were found to lower blood pressure in 24-mo-old Sprague-Dawley but not in Fischer 344 rats (13). Moreover, because our previous studies showed that intravenously injected lisinopril reduced angiotensin-induced reflex bradycardia only at 19 but not at 4 mo of age (28), it seemed likely that ACE inhibitors would also have age-related baroreflex effects in Sprague-Dawley rats. However, whether chronic treatment with lisinopril has similar age-related effects on baroreflex sensitivity and pressor responsiveness has yet to be determined. Pressor responsiveness is likely to be differently affected in old rats because both the activity of the renin-angiotensin system (1) and the cardiovascular effects of adrenergic drugs (6) diminish with increasing age.

Accordingly, the present studies aimed to compare the effects of chronic lisinopril treatment on baroreflex sensitivity and pressor responsiveness in two age groups of conscious Sprague-Dawley rats. Half of the rats in each age group had lisinopril added to their drinking water, whereas the others, serving as untreated controls, drank tap water alone. A daily dose of 1.0–1.5 mg/rat (or 2.5 mg/kg) of lisinopril was given for 30 days because this dose inhibited ACE activity and improved aortic distensibility in normotensive rats, even when arterial pressure was unaffected (27). Indwelling vascular catheters were chronically implanted to allow recording of cardiovascular drug effects while the rats were conscious. Baroreflex sensitivity was assessed by measuring phenylephrine-induced reflex bradycardia, whereas pressor responsiveness was assessed, after heart rate reflexes had been blocked, by measuring the blood pressure increases elicited with angiotensin II or norepinephrine.
METHODS

Forty-four male Sprague-Dawley rats were purchased from SASCO (Omaha, NE) at ages of either 4 or 21 mo. On the basis of the age difference, the 4-mo-old rats (n = 24) will henceforth be referred to as "young," whereas the 21-mo-old rats (n = 20) will be referred to as "old". Half of the rats in each age group were treated with lisinopril, whereas the rest served as untreated controls. During the 30-day period of lisinopril treatment, indwelling catheters for cardiovascular recording were implanted into each rat on day 21, and tests were recorded for baroreflex sensitivity on day 28 and for pressor responsiveness on day 30. Subsequent data analyses compared four rat groups classified as 1) young untreated (n = 12), 2) young lisinopril treated (n = 12), 3) old untreated (n = 10), and 4) old lisinopril treated (n = 10). Because technical difficulties resulting from either anesthesia or nonfunctional catheters precluded completion of all recordings, experiments that had questionable blood pressure responses were discarded, and data analysis was limited to only 22 young and 19 old rats for baroreflex sensitivity and to 21 young and 17 old rats for pressor responsiveness.

Chronic lisinopril treatment for 1 mo. Daily fluid intake expressed as milliliters per 24 hours was determined by weighing the water bottle from each rat cage at the same time every morning and calculating the reduction of bottle weight in grams. Because previous studies had shown that old male Sprague-Dawley rats are ~30% heavier than young ones (7), lisinopril concentrations per 100 milliliters of drinking water were adjusted to contain 15 mg for old rats and 10 mg for young rats. Based on a daily fluid intake of 10 ml/rat, these drug solutions were estimated to deliver a lisinopril dose of 2.5 mg·kg⁻¹·day⁻¹ for both age groups during the 30-day period of chronic treatment.

Surgical implantation of indwelling cannulas. To allow recording of baroreflex and pressor drug responses from conscious rats, indwelling femoral cannulas were implanted chronically 1 wk before baroreflex testing. Each rat was transiently anesthetized with pentobarbital sodium (5.5 mg/100 g ip), and indwelling cannulas filled with heparinized saline (30 U/ml) were inserted separately into the left femoral artery for blood pressure recording and into the ipsilateral vein for drug infusions (7). Cannulas consisted of two pieces of polyethylene tubing: a 3- to 5-cm length of PE-10 threaded and heat-fused into a 13- to 18-cm length of PE-50. The inner end of PE-10 tubing was inserted into the artery or vein, whereas the outer end of PE-50 tubing was tunneled subcutaneously, exteriorized at the nape, and sealed with a 23-gauge plug. To allow complete postoperative recovery, all rats were left untouched for 7 days in individual plastic cages in an air-conditioned room with unrestricted access to food (rodent laboratory chow; Ralston Purina, St. Louis, MO) and water.

Cardiovascular recording of drug responses in conscious rats. For the two recording sessions on days 28 and 30, each rat was kept in its own open-topped plastic cage throughout the recordings. Pulsatile pressure was recorded by connecting the arterial cannula through PE-50 tubing to a small-volume-displacement pressure transducer (P10 E2; SpectraMed, Oxnard, CA) placed outside the cage at the same level as the rat. Analog signals for mean arterial pressure (calculated as diastolic pressure plus one-third of the pulse pressure) and heart rate were derived from the pulsatile pressure signal with the use of a cardiovascular analyzer (CVA-1; Buxco Electronics, Sharon, CT). After connecting the rat to the recording apparatus, the rat was left undisturbed for 30 min to allow blood pressure and heart rate to return to resting levels.

To assess baroreflex sensitivity during the first recording session on day 28, reflex bradycardia was elicited by using graded intravenous infusions of phenylephrine to elevate systemic pressure. Phenylephrine was infused by connecting the indwelling venous catheter through PE-50 tubing to a 6-ml plastic syringe mounted on an infusion pump (model 22; Harvard Apparatus, South Natick, MA) driven by a computer. As described previously (7), a graded infusion ramp was produced by programming the computer to increase infusion rates from 23 to 117 µl·min⁻¹·100 g body wt⁻¹ in 50 steps (each lasting 1.0 s). After total volumes of 200–400 µl/100 g over 5 s were infused, phenylephrine doses ranging from 0.6 to 3.0 µg·min⁻¹·100 g⁻¹ were delivered to elevate mean pressure by 40–50 mmHg.

Two days later on day 30, a second recording session was performed to assess pressor responsiveness after combined cholinergic and β-adrenergic blockade had been induced. All rats were first pretreated with propranolol (1 mg/kg initially and 0.5 mg/kg 45 min later) to induce β-adrenergic blockade and with atropine (0.5–1.0 mg/kg initially and 0.25 mg/kg 45 min later) to induce cholinergic blockade. The combined blockade thus produced has been shown to abolish the reflex bradycardia elicited with norepinephrine (5). Pressor responsiveness was then measured by recording increases in mean arterial pressure produced by injecting graded doses (in ng/100 g) of 3, 6, and 10 for angiotensin and of 10, 16, and 26 for norepinephrine.

Data acquisition and analysis. A data acquisition system consisting of a personal computer, 12-bit analog-to-digital board (DT 2801; Data Translation, Marlboro, MA), WFS hardware recorder, and programs for data acquisition (DATAQ Instruments, Akron, OH), was used to digitize analog outputs from the cardiovascular analyzer (7). Digitized data were stored in files from which corresponding units in millimeters of mercury for mean pressure and in beats per minute for heart rate were determined. Before each recording, pressure calibrations were checked and zero levels were adjusted with a mercury manometer. Data points obtained from each recording by reading mean pressure and heart rate every second during infusions of phenylephrine or angiotensin were smoothed with the Blackman window filter function with a cutoff frequency of 0.1 Hz. Values recorded immediately before infusions began were averaged to obtain preinfusion baseline levels, whereas those recorded for 50 s during infusions were used to calculate drug-induced reflex responses. Data thus obtained were analyzed first by averaging mean pressure and heart rate changes produced by infusing progressively increasing drug doses and then by calculating linear regression slopes.

Drugs, doses, and statistics. Drug doses, except for lisinopril, are expressed as the amount of the salt per 100 grams body weight. For eliciting reflex bradycardia, phenylephrine hydrochloride (Elkins-Sinn, Cherry Hill, NJ) was infused intravenously, whereas for pressor responsiveness angiotensin II acetate (Sigma, St. Louis, MO) or norepinephrine bitartrate (Levophed; Sandi Winthrop Pharmaceuticals, New York, NY) were given as single bolus injections. Atropine methyl nitrate and dl-propranolol hydrochloride (both from Sigma) were used to induce combined cholinergic and β-adrenergic blockade before tests of pressor responsiveness. Lisinopril was generously supplied by Merck Research Laboratories (Rahway, N J ).

All data are expressed as means ± SE. Baseline values for mean pressure, heart rate, and body weight were compared using a general measures ANOVA (3). To eliminate variations caused by different time points of measurement during baroreflex tests involving infusions of phenylephrine, drug-
induced changes in mean pressure and heart rate were compared using a multivariate ANOVA to obtain F ratios for group and time effects. Correlations between baseline mean pressure and height of pressor responses to angiotensin II and norepinephrine were obtained by calculating Pearson product-moment correlation coefficients with corresponding P values (Table 5). Percent changes in pressor responses (Table 6) were ranked, and a multivariate ANOVA was used on both ranked and percent values. If both analyses gave similar results, then distribution of percent changes was assumed to be normal, and a repeated-measures ANOVA was performed on the percent changes. Whenever F ratios significant at 5% or less were obtained, a Newman-Keuls multiple range test was used to determine the significance of differences between pairs of means (3). All statistical comparisons and tests were done using the NCSS 6.0 Statistical System for Windows (18).

RESULTS

Lisinopril does not affect body weight or fluid intake. As described previously (7), old male Sprague-Dawley rats were invariably larger and heavier than the young rats throughout our studies. Average body weight during the 4-wk treatment period remained essentially unaltered in old rats but increased progressively in young rats (Table 1). In both age groups, rats treated with lisinopril always weighed less than untreated controls of the same age, but ensuing differences in average body weight within each age group were not statistically significant. Old rats initially tended to drink less than the young ones, and average fluid intake was slightly increased on addition of lisinopril (Table 1), but the differences between treated and untreated rats of the same age were statistically significant only during the first week. Hence, these results indicate that, despite initial differences between age groups, chronic oral treatment with lisinopril in daily doses of 1.0–1.5 mg/rat (or 2.5 mg/kg) for 3 wk did not affect either body weight or fluid intake.

Table 1. Changes in body weight and fluid intake during first 3 wk of daily lisinopril treatment in young or old rats

<table>
<thead>
<tr>
<th>Time, wk</th>
<th>Young untreated (n=12)</th>
<th>Young lisinopril (n=12)</th>
<th>Old untreated (n=10)</th>
<th>Old lisinopril (n=10)</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>374±7</td>
<td>366±6</td>
<td>623±18</td>
<td>596±14</td>
<td>155.42</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>2</td>
<td>399±8</td>
<td>388±8</td>
<td>625±14</td>
<td>599±13</td>
<td>149.96</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>3</td>
<td>428±8</td>
<td>412±9</td>
<td>625±13</td>
<td>595±13</td>
<td>107.43</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>4</td>
<td>439±8</td>
<td>415±9</td>
<td>620±12</td>
<td>596±13</td>
<td>98.18</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

Values for body weight and fluid intake are means ± SE; n = no. of rats. F ratio for group differences obtained by multivariate ANOVA. *P < 0.05 compared with corresponding averages for young rats by Newman-Keuls multiple-range test.

Table 2. Cardiovascular baselines before baroreflex and pressor tests in young or old rats pretreated with lisinopril

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>Young untreated (n=12)</th>
<th>Young lisinopril (n=12)</th>
<th>Old untreated (n=10)</th>
<th>Old lisinopril (n=10)</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10.5±0.9</td>
<td>12.7±0.9</td>
<td>9.1±0.9</td>
<td>9.3±0.9</td>
<td>3.53</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>2</td>
<td>10.0±0.8</td>
<td>11.0±0.8</td>
<td>9.0±0.9</td>
<td>9.5±0.9</td>
<td>1.36</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>3</td>
<td>10.8±1.0</td>
<td>11.6±1.0</td>
<td>9.3±1.0</td>
<td>9.5±1.1</td>
<td>1.59</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>4</td>
<td>10.1±1.0</td>
<td>10.6±1.0</td>
<td>9.3±1.0</td>
<td>9.5±1.1</td>
<td>1.39</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

Values for mean blood pressure (BP) and heart rate are means ± SE; n = no. of rats. F ratio for group differences obtained by multivariate ANOVA. *P < 0.05 compared with corresponding averages for young rats by Newman-Keuls multiple-range test.

Lisinopril lowered blood pressures without affecting heart rates. Baseline averages for mean arterial pressure and heart rate were obtained twice during the 4-wk treatment period, on days 28 (i.e., before testing baroreflex sensitivity) and 30 (i.e., before testing pressor responsiveness), respectively. Heart rates recorded on day 28 did not differ between age groups whether treated with lisinopril or not. Mean pressures were, however, always higher in old rats than they were in young rats (Table 2). Thus on day 28 the average mean pressure of 134 ± 4 mmHg in old untreated controls was 20 mmHg higher than that of 114 ± 4 mmHg in young untreated controls. With the hypotensive effect produced by lisinopril treatment, the difference between age groups was only 14 mmHg as average mean pressures fell to 107 ± 5 mmHg in old and 93 ± 2 mmHg in young rats. Lisinopril was equally effective in lowering blood pressure in both age groups, inasmuch as the ensuing percent reductions in mean pressure were −18.4% in young and −20.1% in old rats (P > 0.1).

Subsequent induction of combined cholinergic and β-adrenergic blockade 2 days later (before testing pressor responsiveness on day 30) elevated mean pressures slightly in all rat groups (Table 2), but the averages still remained significantly higher in old rats than in young rats. In the presence of combined blockade, hypotensive effects of lisinopril still did not differ between age groups (i.e., −18.2% in young vs. −17.6% in old rats; P > 0.1). Heart rates, although reduced by combined blockade selectively in old but not in young rats, were unaffected by lisinopril treatment and always remained significantly lower in old rats than they did in young rats, regardless of whether lisinopril had been given or not.

Reflex bradycardia enhanced by lisinopril more in young rats. During baroreflex tests with intravenous infusions of phenylephrine, ensuing elevations in mean
pressure consistently elicited reflex bradycardia whether the rats were young or old. In untreated control rats, magnitude of pressor and bradycardic responses plotted against the duration of phenylephrine infusion increased progressively at 5-s intervals (Table 3), but magnitude of either response was not statistically different between age groups. With lisinopril treatment, bradycardic responses to phenylephrine seemed larger in both age groups, but the group differences were not significant (F ratio 0.66, P > 0.5).

To quantify the effects of lisinopril on the level of baroreflex sensitivity, the reduction in heart rate was plotted against every 5-mmHg increment during the 40-mmHg increase in mean pressure (Fig. 1) instead of being greater in young rats than it was in old rats (Fig 1).

Pressor responses to angiotensin II and norepinephrine enhanced by lisinopril, particularly in young rats. Combined cholinergic and β-adrenergic blockade was then induced in the same rats 2 days later to allow measurement of pressor responses that had not been buffered by compensatory heart rate reflexes. Consequently, subsequent intravenous injections of angiotensin II or norepinephrine elevated mean pressures with-out appreciable changes in heart rate. Regardless of rat grouping, height of all ensuing pressor responses to either drug was dose dependent, and the magnitude of blood pressure elevations elicited increased progressively with increasing doses. Pressor responses to both drugs, expressed as absolute increases in millimeters of mercury of mean pressure, were consistently smaller in old rats than they were in young rats, with all differences between age groups being statistically significant (Table 4). Pressor responses, particularly to angiotensin II, were often smaller in lisinopril-treated rats, but none of the differences between untreated and lisinopril-treated rats within the same age group were significant because baseline pressures were initially higher in old rats than they were in young rats but were lowered almost equally in both age groups by lisinopril (Table 2). It seemed possible that baseline differences between rat groups could have influenced the responses to the pressor drugs.

To determine whether any correlation exists between baseline pressure levels, mean pressures recorded immediately before each drug injection were paired with the peak elevation in mean pressure. Paired values for all drug injections were then pooled, and Pearson correlation coefficients were calculated by plotting the two variables against each other (Table 5). All of the correlation coefficients thus obtained, whether calculated for each pressor drug alone or for both drugs together, were highly significant (P < 0.00001). These results therefore indicate that magnitude of pressor responses in general (i.e., for either angiotensin II or

**Table 3.** Pressor and bradycardic responses to intravenously infused phenylephrine in young or old rats pretreated with lisinopril

<table>
<thead>
<tr>
<th>Infusion Duration, s</th>
<th>Rat Groups</th>
<th>Pressor response, mmHg</th>
<th>Reflex bradycardia, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young untreated</td>
<td>Young lisinopril</td>
<td>Old untreated</td>
</tr>
<tr>
<td>5</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>10</td>
<td>7 ± 1</td>
<td>4 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>15</td>
<td>18 ± 2</td>
<td>11 ± 1</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>29 ± 2</td>
<td>19 ± 2</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>25</td>
<td>38 ± 2</td>
<td>27 ± 2</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>30</td>
<td>44 ± 2</td>
<td>33 ± 2</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>35</td>
<td>47 ± 2</td>
<td>37 ± 2</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>40</td>
<td>50 ± 2</td>
<td>40 ± 2</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>45</td>
<td>52 ± 2</td>
<td>42 ± 2</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>50</td>
<td>53 ± 2</td>
<td>43 ± 2</td>
<td>48 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rat groups are same as presented in Tables 1 and 2.
Table 4. Pressor responses produced by injecting graded doses of angiotensin II or norepinephrine in 2 age groups of conscious rats after 30 days of lisinopril treatment

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>Pressor Drug Dose, ng/100 g</th>
<th>Young untreated (n = 12)</th>
<th>Young lisinopril (n = 10)</th>
<th>Old untreated (n = 7)</th>
<th>Old lisinopril (n = 10)</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressor responses to angiotensin, mmHg</td>
<td>3</td>
<td>35 ± 2</td>
<td>31 ± 3</td>
<td>25 ± 3*</td>
<td>21 ± 1*</td>
<td>7.43 &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>40 ± 2</td>
<td>36 ± 4</td>
<td>29 ± 3*</td>
<td>24 ± 1*</td>
<td>7.97 &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>56 ± 2</td>
<td>52 ± 3</td>
<td>46 ± 5*</td>
<td>39 ± 2*</td>
<td>7.52 &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values for pressor responses are means ± SE and are expressed as absolute increases in mean pressure; n = no. of rats. **ANOVA. *P < 0.05 compared with corresponding averages for young rats, whether untreated or lisinopril-treated, by Newman-Keuls multiple-range test.**

DISCUSSION

Our most compelling new finding here is that oral treatment with lisinopril, 1.0—1.5 mg/rat (or 2.5 mg/kg) daily for 4 wk, enhanced reflex bradycardia as well as pressor responsiveness to angiotensin II and norepinephrine in male Sprague-Dawley rats. Both effects were more pronounced in young rats than they were in old rats. Hence, phenylephrine-induced reflex bradycardia was stronger in lisinopril-treated rats than it was in untreated rats of the same age (Table 3) and also more marked in young rats than it was in old rats (Fig. 1). On the other hand, lisinopril effects on pressor responsiveness to angiotensin II and norepinephrine in young rats, the enhancement produced in old rats was equivocal because it was limited only to the 16 ng/100 g dose of norepinephrine.

**Table 5. General correlation between baseline mean BP and magnitude of pressor responses to angiotensin II, norepinephrine, or both drugs combined**

<table>
<thead>
<tr>
<th>Drug Group</th>
<th>Mean BP, mmHg</th>
<th>Pearson's Correlation Coefficient</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Angiotensin</td>
<td>114</td>
<td>117 ± 2</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>113</td>
<td>120 ± 2</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Both drugs</td>
<td>227</td>
<td>119 ± 1</td>
<td>32 ± 1</td>
</tr>
</tbody>
</table>

Values for mean BP are means ± SE; n = no. of injections.

**Table 6. Pressor responses produced by injecting graded doses of angiotensin II or norepinephrine in 2 age groups of conscious rats after 30 days of lisinopril treatment**

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>Pressor Drug Dose, ng/100 g</th>
<th>Young untreated (n = 12)</th>
<th>Young lisinopril (n = 9)</th>
<th>Old untreated (n = 7)</th>
<th>Old lisinopril (n = 10)</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressor responses to angiotensin, %</td>
<td>3</td>
<td>24 ± 2</td>
<td>32 ± 2</td>
<td>37 ± 2*</td>
<td>13 ± 3*</td>
<td>16 ± 2*</td>
<td>14.11 &lt; 0.00001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>28 ± 2</td>
<td>37 ± 3*</td>
<td>16 ± 3*</td>
<td>19 ± 2*</td>
<td>14.27 &lt; 0.00001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>41 ± 4</td>
<td>59 ± 5*</td>
<td>27 ± 5*</td>
<td>33 ± 4*</td>
<td>8.74 &lt; 0.0002</td>
<td></td>
</tr>
</tbody>
</table>

Values for pressor responses are means ± SE and are expressed as percent increases in mean BP calculated from baseline pressures for each group in Table 2 (before pressor responsiveness test); n = no. of rats. **ANOVA. *P < 0.05 compared with corresponding averages for young untreated rats by Newman-Keuls multiple-range test; †P < 0.05 compared with corresponding average for young lisinopril-treated rats by Newman-Keuls multiple-range test; ‡P < 0.05 compared with corresponding average for old untreated rats by Newman-Keuls multiple-range test.**
and increased pressor responsiveness) were more marked in young rats, these results overall suggest
that the cardiovascular effects produced by lisinopril diminish with advancing age.

The enhancement of reflex bradycardia by lisinopril seen here confirms the baroreflex enhancement previously described for various ACE inhibitors in normoten-
sive (11) or hypertensive (12) patients as well as in SHR (2, 23, 24). Contrasting with the 4-wk regimen we used
on normotensive Sprague-Dawley rats, oral lisinopril treatment for only 1 wk enhanced baroreflex gain
selectively in SHR but not in normotensive Wistar-Kyoto (WKY) rats (24). Theoretically, ACE inhibitors
increase baroreflex sensitivity mainly by reducing angiotensin formation so that there is less baroreflex
inhibition by angiotensin at central or peripheral sites of the reflex arc (14). On the contrary, however, instead
of showing enhanced reflex bradycardia, our earlier studies showed that reflex bradycardia was reduced by intravenously injected lisinopril (28). Considering those
earlier studies together with our present results, opposite effects on reflex bradycardia have been produced
such that reflex bradycardia was enhanced when lisinopril was given orally for 4 wk (Fig. 1) but inhibited
when single bolus injections were given intravenously. This discrepancy must be partly a result of differences
in availability of lisinopril at its sites of action. Compared with the daily 2.5 mg/kg (i.e., 1.0–1.5 mg/rat)
oral dose used here, the 10 mg/kg iv dose used earlier was four times larger and must have delivered suffi-
ciently high drug levels to depress some part of the baroreflex arc.

Baselines for blood pressure often vary extensively in
rat models for aging, obesity, or diabetes (4) because the
trat populations studied usually consist of an irregular
assortment of normotensive and hypertensive rats. To
evaluate pressor responsiveness in such mixed popula-
tions, the different baselines can be normalized by
expressing the drug-induced blood pressure increases
in each rat as percent increases from the respective
baseline. If ensuing group differences in responsiveness
are significant only when analyzed as absolute in-
creases but not when analyzed as percent increases,
then they are probably not real increases in pressor
responsiveness but simply artefacts caused by baseline
differences. On the other hand, group differences that
remain statistically significant even when analyzed as
percent changes are probably real because they persist
even after the baselines have been normalized. As an
example, we were able to identify the age-related
enhancement of reflex bradycardia by ketanserin be-
cause the regression slopes comparing different age
groups remained statistically significant whether the
data were analyzed as absolute or as percent changes
(10).

At first glance our results seem to imply that lisino-
pril reduced pressor responsiveness just like other ACE
inhibitors do. Hence, many of the increases in mean
pressure produced by graded doses of angiotensin or
norepinephrine were slightly reduced by lisinopril in
both age groups (Table 3). These comparisons, however,
completely ignore the differences in baseline pressures,
which resulted because the baselines were always
initially higher in old rats than they were in young rats
and then lowered subsequently by lisinopril in both
groups (Table 2). According to the “law of initial value”
proposed by Wilder (35) for quantifying biological stimu-
lus-response relationships, responses to function-
raising stimuli are smaller as initial values become
higher. Wilder’s law was never validated, but it has
been applied to epinephrine-induced pressor responses
in anesthetized cats (22) or dogs (17). Concurring with
it now, our data for angiotensin and/or norepinephrine
in conscious rats clearly show significant inverse corre-
lations between baseline pressure and pressor re-
sponses (Table 4). Because of this, and also because
baselines varied widely among our different rat groups
(Table 2), the smaller pressor responses elicited here in
old rats (Table 3) are likely a result of their higher
baselines and, if so, then actual changes in responsive-
ness can be determined only after baseline differences
have been normalized. But exactly what the underlying
mechanisms may be (i.e., how higher blood pressure
baselines lead to smaller pressor responses) still re-
 mains conjectural.

Annulling baseline effects on pressor responsiveness
by using percent changes will require not only addi-
tional calculations but also more cumbersome statisti-
cal analyses. The percent values have to be ranked to determine
whether the numbers still follow a normal distribution,
and if not, then nonparametric statistics will be needed.
Nonetheless, the added manipulation in the present
study allowed us to reach a conclusion that would not
otherwise be possible, because the percent changes clearly show two significant group differences: first,
that pressor responses to either drug were consistently
smaller in old rats than they were in young rats; and
second, that although lisinopril increased pressor re-
sponses to both drugs at either age, the increase was
much more pronounced in young rats. The ensuing
conclusion about enhancement by lisinopril, however,
contradicts previous reports on reduction of pressor
responsiveness by ACE inhibitors in humans (15, 19,
26) or anesthetized rats (32).

Discrepancies between our studies and those re-
ported previously by others could in part be a result of differences in animal species or ACE inhibitors. In
clinical studies on human patients, pressor responsive-
ness was reduced by treatment with either captopril
(15, 19, 26) or enalapril (26). However, although the
clinical studies resemble ours in not using anesthesia,
humans obviously differ from rats, and captopril and
enalapril are not exactly identical to lisinopril. For
instance, lisinopril differs structurally from captopril
in that it does not contain a sulfhydryl group and differs
from enalapril in that it is not an ester prodrug (8).

The only other relevant study on pressor responsive-
ness was that by Spertini et al. (32), but although they
also used rats, our methods and results differ in many
ways. Our rats were conscious Sprague-Dawley males
pretreated with lisinopril (1 mg/kg daily) for 4 wk,
whereas theirs were anesthetized albino females pre-
treated with captopril (100 mg/kg twice daily) for 14 days. Moreover, we found pressor responses to both angiotensin and norepinephrine were enhanced, whereas they found responses only to angiotensin but not to norepinephrine were enhanced. In discussing their results they considered baseline influences on pressor responsiveness unlikely because only responses to angiotensin were enhanced selectively. This explanation seems dubious, however, in light of the fact that their baseline differences, which were much larger than ours (i.e., hypotensive effects of captopril in their rats ranged from −38 to −58 mmHg compared with −11 to −27 mmHg produced by lisinopril in ours), would have been more likely to affect pressor responsiveness than they would have been in the present studies.

An alternative explanation for our findings might be that doses of lisinopril differed between age groups over the 30-day treatment period. Kumagai et al. (23) found that oral lisinopril treatment (10 mg/kg daily added to drinking water for 1 wk) lowered mean arterial pressure selectively in SHR but not in WKY rats. In the present studies, lisinopril concentrations in drinking water were adjusted from 10 mg/100 ml in young rats to 15 mg/100 ml in old rats to compensate for the larger body weights of old rats, but old rats tended to drink less fluid, and their fluid intake was significantly lower during the first week (Table 1). Were this to persist, the old rats would then absorb less lisinopril simply because they did not drink as much each day. Although adjusted concentrations would have delivered a daily dose of 2.5 mg/kg had all rats drunk 10 ml/day, whenever old rats drank less than that they received smaller doses than the young rats. For instance, the daily consumption of lisinopril for a young rat drinking 10 ml/day of a 10 mg/100 ml solution would be 1 mg, whereas that for an old rat drinking 9 ml/day of a 15 mg/100 ml solution would be 1.35 mg. Based on average body weights of ~400 g for young rats and ~600 g for old rats (Table 1), estimated daily doses in milligrams per kilogram would then be 2.5 and 2.25, respectively. Effects of lower doses for old rats could in part be corrected by age-related reductions in hepatic metabolism or renal excretion, like those that occur in the elderly. However, because group differences in fluid intake were no longer statistically significant after the first week (Table 1), the possibility that old rats may have received lower doses of lisinopril is negligible. In retrospect, the dose differences between age groups could have been avoided here by using chronically implanted osmotic minipumps to administer lisinopril treatment.

Finally, perhaps the most important distinguishing feature of our studies was the routine use of combined cholinergic and β-adrenergic blockade to eliminate all heart rate reflexes. Because cardiovascular homeostasis normally counteracts disturbances in blood pressure, the height of any drug-induced elevation in blood pressure is usually reduced by lessening cardiac activity through reflex bradycardia, and pressor responses to norepinephrine and other test drugs would then be inhibited. By contrast, because compensatory heart rate reflexes in our rats were prevented by combined cholinergic and β-adrenergic blockade, the pressor responses elicited with angiotensin or norepinephrine were not buffered by reflex bradycardia and were mostly caused, therefore, by vasoconstriction.

In summary, our results show that chronic oral lisinopril treatment enhanced both reflex bradycardia and pressor responsiveness in conscious male rats. Of these two effects, enhanced reflex bradycardia could work to more effectively compensate for the blood pressure fall produced by lisinopril. On the other hand, enhanced pressor responsiveness, which would tend to elevate, rather than lower, blood pressure, could be potentially harmful when lisinopril is used clinically for antihypertensive treatment. Despite the conceivably hazardous effect of the pressor enhancement produced by lisinopril, however, its reduction in old rats may explain, at least in part, why ACE inhibitors can still lower blood pressure effectively despite the low plasma renin levels in elderly hypertensives.

Perspectives

To study the cardiovascular effects of chronic oral lisinopril treatment, we compared baroreflex sensitivity and pressor responsiveness in 4-mo- and 21-mo-old male rats that had been given oral lisinopril daily for 4 wk. Reflex bradycardia was clearly enhanced by lisinopril treatment in both age groups, but lisinopril effects on pressor responsiveness were ambiguous. Pressor responses to angiotensin or norepinephrine, expressed as absolute increases in mean pressure, were slightly increased by lisinopril in both age groups. But when expressed as percent increases to compensate for group differences in baseline pressures, pressor responses were clearly intensified by lisinopril in young rats but probably not in old rats. Because combined cholinergic and β-adrenergic blockade was used routinely to eliminate all heart rate reflexes during tests for pressor responsiveness, the pressor responses elicited with angiotensin or norepinephrine were not buffered by reflex bradycardia and were mostly caused by vasoconstriction. The stronger overall enhancement of pressor responsiveness and reflex bradycardia in younger rats suggests that the cardiovascular effects of lisinopril abate with advancing age.

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