β-Adrenoceptor-Stimulated Renin Release Is Blunted in Old Rats

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Abstract. Plasma renin activity (PRA) was similar in young versus old male Sprague Dawley rats under unstressed conditions (1.3 ± 0.2 versus 1.8 ± 0.3 ng angiotensin I/ml per min). Airjet stress increases PRA in young but not old rats (13.9 ± 3.8 versus 2.9 ± 0.8 ng angiotensin I/ml per min), respectively. This response is ablated in young rats by β-adrenoceptor blockade, suggesting that the increased PRA is mediated by β-adrenoceptors, and this response was blunted in old rats.

It is generally accepted that plasma renin activity (PRA) declines with advancing age in several species, including humans and rats (1–5). However, we recently showed that in the conscious chronically catheterized rat, basal PRA was similar in young adult (3 to 5 mo old) and old (18 to 20 mo old) rats (6). Although values in old rats were similar to those published previously, PRA in young rats was lower in our conscious, unstressed preparation versus others (1–5,7). Elevated values of PRA indicate volume depletion and/or anesthetic, surgical, or emotional stress. It is possible that the higher values of PRA reported in young rats reflect stimulation due to one or more stressors. If so, the blunting or absence of a similar stress response in old animals could explain the apparent reduction in PRA with aging. To investigate this possibility, the present study was conducted in young and old, conscious, chronically catheterized rats to determine whether stress-induced, β-adrenoceptor-dependent renin release diminishes with advancing age.

Materials and Methods

Studies were conducted in male Sprague Dawley rats (Harlan, Indianapolis, IN) 3 to 5 mo (young, n = 6) or 18 to 20 mo (old, n = 6) of age, maintained on chow ad libitum (sodium, 0.31%; protein, 24%) and tap water. Under short-acting barbiturate anesthesia (Brevital; Eli Lilly, Inc., Indianapolis, IN) and using full sterile technique, vascular catheters were placed in the femoral artery and vein, as described previously (8). Rats were allowed at least 7 d for recovery from surgery, anesthesia, or both, and during this time they were handled and trained to accustom them to activities in the laboratory.

Experiments were conducted as follows. Rats were placed in a restraining cage, the arterial and venous lines were opened, and BP and heart rate (HR) were measured throughout the study (Maclab, ADI Instruments, Milford, MA). After approximately 40 min of equilibration, when the rat was calm and BP was stable, a control arterial blood sample was taken (1 ml, withdrawn slowly) and was rapidly centrifuged, and the red cells were reconstituted with sterile 13.4% Ficoll solution and returned to the rat. The whole blood was collected with 20 μl of ethylenediaminetetra-acetic acid (75 mg/ml) and spun at 4°C, and 500 μl of plasma was aliquoted and stored at −20°C until analysis. BP and HR were measured for approximately 10 min after restoration of blood until values returned to presampling levels. Next, rats were airjet-stressed (AJS) for 10 min, and a 1-ml blood sample was taken during the last 30 s of AJS. AJS provides a rapid and reversible activation of the sympathetic nervous system (SNS), as described previously (9,10). Briefly, a continuous, hard stream of air was blown into the rat’s face for 10 min through a space at the front of the restraining cage, and the combination of air flow and sound produced considerable agitation. This method of stress has been reported to stimulate renin release (9), elevate BP, and increase renal sympathetic nerve discharge in conscious rats (10). Although there was no way to quantify the subjective response of the rats to AJS, both young and old rats in the present study responded with obvious agitation and attempts at evasive behavior (i.e., moving head from side to side). Two experiments (order randomized) were performed on each rat, once in the baseline state and once during β-adrenoceptor blockade with propranolol (1.35 × 10−6 M; intravenously, approximately 25 min before the control blood sample). This dose completely blocks the depressor response to 10−6 M of isoproterenol (11). At least 3 d elapsed between the first and second experiments.

PRA was determined by RIA of angiotensin I, using a GammaCoat kit (Incstar, Stillwater, MN) as adapted from the original method of Haber (12).

Statistical Analyses

Data were expressed as mean ± SEM. Statistical analyses were by paired t test within a group, and by repeated measures ANOVA for between-group comparisons. Statistical significance is defined when P < 0.05.

Results

Body weight was greater in old versus young rats (539 ± 27 versus 421 ± 15 g; P < 0.01). As shown in Table 1, in unstressed conscious rats, baseline PRA was low and was similar in young and old rats. When the SNS was intact, AJS increased PRA eightfold in young rats, and BP and HR rose dramatically. In contrast, in old rats AJS produced a nonsignificant change in PRA, whereas BP remained unchanged and
The reduced PRA and HR response to AJS in old rats suggests a blunted β-adrenergic response with age, and there is evidence of widespread downregulation of the β-adrenergic receptors with aging (13). The limitations of our whole animal model do not allow us to discriminate the exact nature of the decreased β-adrenergic-mediated renin response in the old rats. Nevertheless, the blunted β-adrenergic response in old rats has been widely studied and is apparently predominantly due to a defective G protein coupling of the receptor to the adenylyl cyclase (13), which suggests a reduced juxtaglomerular cell response to β-adrenergic stimulation. However, reductions in receptor density cannot be ruled out, because this finding has also been reported in a variety of locations with age (13). A defective β-adrenergic response in the old rats is supported by our finding that PRA did not change in response to AJS during β-adrenergic blockade in both age groups. We and others have observed that stimulation of PRA by angiotensin-converting enzyme inhibition is diminished (4,6) and that the increase in PRA in response to dietary sodium restriction is blunted, despite appropriate stimulation of renal renin mRNA in the old rat (14,15). Overall, it seems that with advancing age, although basal levels are not different, renin synthesis/release in response to multiple stimuli is attenuated. This trend would result in lower PRA values in old rats in many situations other than the controlled, conscious basal state.

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References

Table 1. Summary of PRA, BP, and HR responses to airjet stress in conscious, young and old male Sprague Dawley rats

<table>
<thead>
<tr>
<th>Category</th>
<th>PRA (ng Al/ml per min)</th>
<th>BP (mmHg)</th>
<th>HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1.8 ± 0.3</td>
<td>123 ± 2</td>
<td>377 ± 11</td>
</tr>
<tr>
<td>+ stress</td>
<td>13.9 ± 3.8</td>
<td>139 ± 4</td>
<td>497 ± 6</td>
</tr>
<tr>
<td>Old baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1.3 ± 0.2</td>
<td>126 ± 12</td>
<td>396 ± 8</td>
</tr>
<tr>
<td>+ stress</td>
<td>2.9 ± 0.8</td>
<td>130 ± 13</td>
<td>440 ± 9</td>
</tr>
</tbody>
</table>

a PRA, plasma renin activity; Al, angiotensin I; HR, heart rate; bpm, beats per minute.

b Denotes paired difference from control.

c Denotes difference in response to airjet stress in young versus old rats.

d Denotes difference in response to airjet stress with or without propranolol, by two-way ANOVA.

Discussion
The present study confirms our earlier report (6) that PRA is similar in young and old rats when measured in blood sampled from a conscious, unstressed preparation. As suggested previously, this is because the values that we obtained from young rats are substantially lower than other reports in the literature, whereas our values of PRA in old rats are similar to published values (2,4,5,7). In the present study, we have provided an explanation for this disparity. We observe that increases in PRA induced by activation of the SNS are attenuated in old rats. Thus, any method of blood sampling that involves acute surgical stress, anesthesia, volume depletion, and/or emotional stress will preferentially exaggerate PRA in young rats compared with old, suggesting that PRA is apparently reduced by aging.

the increase in HR was only approximately 30% of that observed in the young rats (Table 1).

Administration of the β-adrenergic blocker to conscious rats had no effect on baseline PRA but increased HR and reduced BP, compared with the values measured immediately before β-adrenergic blockade, in all rats (young rats: 124 ± 2 versus 120 ± 2 mmHg, P < 0.05, and 324 ± 7 versus 377 ± 9 beats per min [bpm], P < 0.001; old rats: 139 ± 4 versus 133 ± 5 mmHg, P < 0.05, and 315 ± 7 versus 377 ± 13 bpm, P < 0.005). As shown in Table 1, β-adrenergic blockade prevented the stimulation of PRA by AJS. β-Adrenergic blockade had no effect on the BP response to AJS but, predictably, propranolol attenuated the HR responses by 70 to 75% in both young and old rats.


