Isoproterenol-Evoked Renin Release from the In Situ Perfused Kidney

Dose-Response Characteristics in Spontaneously Hypertensive and Normotensive Wistar Rats

ALAN R. SINAIKO AND BERNARD L. MIRKIN

SUMMARY We studied the dose-response relationship between renin secretion and isoproterenol administration in the in situ perfused kidney of spontaneously hypertensive (SHR) and normotensive Kyoto Wistar (WKY) rats obtained from two breeding facilities (BioLab and Taconic Farms). Following pulse injections of isoproterenol (doses ranging from $5 \times 10^{-9}$ M to $5 \times 10^{-6}$ M), timed samples of perfusate were collected over a 30-minute period and perfusate renin activity (PeRA) in each sample was determined by radioimmunoassay. Analysis of regression lines constructed from data representing the renin secretory response (quantified by plotting PeRA of each sample against time of collection and measuring the area under the curve) to isoproterenol concentration demonstrated a positive dose-response relationship for WKY ($y = 690.87 + 2864.06x$) and SHR ($y = 206.56 + 496.54x$). Regression analysis of data obtained from studies in Taconic Farms and BioLab demonstrated a significant relationship between dose and response. Renin secretion was found to be significantly greater in both WKY strains when comparisons were made between the regression coefficients of WKY and SHR obtained from the same breeding facility (BioLab, $P < 0.0005$; Taconic Farms, $P < 0.025$). This investigation has established that the release of renin from the kidney elicited by isoproterenol follows a dose-response pattern similar to that observed with other adrenergically mediated responses. Furthermore, the response of the WKY to isoproterenol-evoked renin secretion was consistently greater than that observed in the SHR strains.

ACTIVATION of the renin-angiotensin system is regulated, in part, by $\beta$-adrenergic mechanisms. Intra-arterial administration of isoproterenol to the in situ perfused rat kidney, incubation of rat kidney slices in catecholamine-containing medium, and electrical stimulation of isolated canine renal nerves produce an increase in renin release that can be blocked by propranolol.

Traditionally, the reactivity of autonomic effector sys-

From the Division of Clinical Pharmacology, Departments of Pediatrics and Pharmacology, University of Minnesota Medical School, Minneapolis, Minnesota.

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tems has been assessed quantitatively by construction of dose-response curves relating adrenergic agonist concentration to responses such as the positive chronotropic and inotropic activity of the heart, or relaxation of the aorta, gastric fundus, and tracheal smooth muscle. In this investigation, the renin secretory response of the in situ perfused kidney to isoproterenol administration was studied in spontaneously hypertensive (SHR) and normotensive Kyoto Wistar (WKY) rats. Dose-response relationships were compared to determine whether genetic selection influences the reactivity of the renin-angiotensin system to β-adrenergic stimuli.

**Methods**

**Animals**

SHR and WKY male rats were purchased from BioLab or Taconic Farms, Inc. The rats comprising the original breeding stock at each of these facilities were obtained from the National Institutes of Health and were direct descendents of either the SHR strain developed by Oka-moto and Aoki or the WKY control strain. All rats were fed tap water ad libitum and regular rat chow containing 0.42% sodium.

The investigation was initiated with 11 SHR and 12 WKY from BioLab. However, this facility discontinued their breeding program and the study was completed with 21 SHR and 21 WKY purchased from Taconic Farms. Each rat was 12-13 weeks of age at the time of investigation.

**Experimental Model**

Perfusion of the isolated in situ rat kidney was performed as previously described in rats anesthetized with pentobarbital sodium (30 mg/kg, ip). The abdominal vessels and left kidney were exposed and polyethylene cannulas inserted into the aorta and vena cava. These vessels then were ligated just above the left kidney and Krebs-Ringer solution oxygenated with 95% O₂-5% CO₂ maintained at 37°C was delivered into the aortic cannula at a flow rate of 5 ml/min, using a Gilson pulsatile flow pump. All samples of perfusate were collected directly from the vena caval catheter during 30-second collection periods.

After steady state renal perfusion was established, two perfuse samples were collected for the determination of basal perfuse renin activity (PeRA), and a pulse injection of isoproterenol HCl (0.1 ml) was delivered directly into the aortic cannula. Experiments in which BioLab rats were used employed concentrations of isoproterenol ranging from $5 \times 10^{-11}$ to $5 \times 10^{-8}$ M, whereas experiments with Taconic Farms rats employed concentrations ranging from $5 \times 10^{-11}$ M to $5 \times 10^{-8}$ M. Samples of perfusate were collected at 1, 2, 3, 4, 6, 10, 14, 19, 24, and 29 minutes after administration of isoproterenol. All samples were immediately frozen and stored at -20°C until the time of assay.

The systemic blood pressure of each rat was obtained by cannulating the abdominal aorta. Renal perfusion pressures were monitored continuously from the aortic line using a Statham P23DB strain-gauge transducer and Beckman Type RS Dynograph.

**Perfusate Renin Activity (PeRA)**

PeRA was determined by a modification of the radioimmunoassay for kidney renin activity developed in this laboratory. Plasma obtained from sheep nephrectomized 48-72 hours prior to bleeding served as the renin substrate source. The following incubation mixture was used: 0.5 ml of sheep plasma, 0.5 ml of perfusate, 1.0 ml of phosphate buffer (pH 6.5), 0.1 ml of ethylene diaminetetraacetic acid (EDTA) (1 mg), 0.01 ml of 2,3-dimercapto-1-propanol (BAL) (1.0 mg), and 0.01 ml of 8-OH quinoline (0.66 mg). Incubation was carried out at 37°C for 3 hours and terminated by rapid cooling of each incubation flask to 4°C. PeRA was estimated, using 0.05 ml of the incubation mixture (angiotensin I radioimmunoassay kit, New England Nuclear) and expressed as nanograms of angiotensin I/ml incubate per hour (ng A I/ml hr⁻¹). Samples of renal perfusate and nephrectomized sheep plasma did not generate significant amounts of angiotensin I when incubated individually.

**Kidney Renin Activity (KRA)**

The right kidneys of five Taconic Farms WKY and SHR were removed, weighed, and frozen immediately in distilled H₂O (100 ml/g tissue) at -20°C. Renin was isolated by homogenizing each kidney after freezing and thawing three times. The supernatant fluid containing the renin was separated and stored at -20°C until assayed.

Kidney renin activity was determined as previously described. Prior to each assay, the supernatant fluid from the homogenized renal tissue was diluted with distilled H₂O to a concentration equivalent to 400 μg tissue/ml. The following incubation mixture was used: 2 ml of sheep plasma, 0.5 ml of supernatant fluid (equivalent of 200 μg of kidney tissue), 6.5 ml of phosphate buffer (pH 6.5), 0.2 ml of EDTA (2 mg), 0.01 ml of BAL (1.0 mg), and 0.01 ml of 8-OH quinoline (0.66 mg). After incubation at 37°C for 3 hours, KRA was determined by radioimmunoassay and expressed as nanograms of angiotensin I (ng A I/ml incubate hr⁻¹).

**Analysis of Data**

Isoproterenol-evoked renin secretion, expressed as PeRA, was estimated quantitatively by plotting PeRA against time and measuring the area under the curve (see Fig. 1). Regression coefficients, representing the dose-response relationship between isoproterenol and renin secretion, were calculated by the method of least squares, and differences between strains were analyzed by Student's t-test to determine statistical significance.

**Results**

**Body Weight**

Each rat was weighed on the day of investigation. WKY was the heavier strain of rats in each case, when SHR and WKY from the same breeding facility were compared. However, the difference in weight between
ISOPROTERENOL-EVOKED RENIN RELEASE IN RATS/Sinaiko and Mirkin

Figure 1

Representative example of in situ kidney perfusion demonstrating effect of isoproterenol on renal perfusion pressure and renin secretion. The response was quantified by plotting the renin activity (ng A/1 ml hr⁻¹) in each perfusate against time of collection and determining the area under the curve.

SHR and WKY was statistically significant only for the strains obtained from Taconic Farms (BioLab: SHR, 277 ± 5 g (mean ± SEM); WKY, 287 ± 9 g; P > 0.05; Taconic Farms: SHR, 269 ± 4 g; WKY, 293 ± 4 g; P < 0.001). When the weights of the SHR and WKY rats obtained from the two separate facilities were compared, no significant intrastrain differences were found (SHR P > 0.05; WKY P > 0.05).

Systemic Blood Pressure

No significant differences in intra-arterial blood pressure were noted between genetically similar strains of rats (BioLab SHR 178 ± 5 mm Hg, Taconic Farms SHR 170 ± 3 mm Hg, P > 0.05; BioLab WKY 120 ± 5 mm Hg, Taconic Farms WKY 114 ± 2 mm Hg, P > 0.05).

Renal Perfusion Pressure

A typical record of renal perfusion pressure and renin secretory response following administration of isoproterenol to the in situ perfused kidney is presented in Figure 1. When renal perfusions were conducted under control conditions without injection of pharmacological agents, the perfusing pressure remained constant over the entire perfusion period. The addition of isoproterenol resulted in a gradual increase in perfusion pressure over the 30-minute experimental period in both strains of rats. This response was significantly greater in SHR than WKY (Table 1). The mean renal vascular resistance was not substantially different in the BioLab strains at the beginning of the perfusion but reached significantly higher levels in the SHR by the end of the perfusion period.

Perfusate Renin Activity (PeRA)

Basal PeRA was significantly greater in both WKY strains than in their SHR counterparts (Table 2). WKY rats from BioLab were found to have a significantly higher basal PeRA, compared with WKY rats from Taconic Farms; however, no significant difference was observed when basal PeRA was compared between Taconic Farms and BioLab SHR.

Determination of the renin secretory response to isoproterenol concentration was limited in the BioLab SHR and WKY rats to two data points for some isoproterenol doses when BioLab unexpectedly discontinued breeding these two strains of rats. However, multifactorial analysis of regression lines constructed from these data (Table 3, Fig. 2) confirmed a significant dose-response relationship for WKY (y = 690.87 + 2864.06x, P < 0.05) and SHR (y = 206.56 + 496.54x, P < 0.05) rats obtained from this source. In contrast, regression analyses of data obtained from the studies using Taconic Farms WKY and SHR demonstrated a significant dose-response relationship only for the WKY strain (y = 138.35 + 74.39x, P < 0.05), but not for the SHR strain (y = 65.15 + 0.95x, P > 0.05).

Renin secretion was significantly greater in both strains of WKY rats when the regression coefficient of each of these groups was compared with that calculated for the SHR group from the same breeding facility (BioLab: P < 0.0005; Taconic Farms: P < 0.01). A significant difference

### Table 1 Renal Perfusion Pressures (Mean ±SEM) at Initiation and Termination of 30-Minute Isoproterenol perfusion Period

<table>
<thead>
<tr>
<th>Rats</th>
<th>Initial (mm Hg)</th>
<th>Terminal (mm Hg)</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taconic Farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (21)*</td>
<td>90 ± 8</td>
<td>121 ± 8</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>WKY (21)*</td>
<td>67 ± 7*</td>
<td>92 ± 8*</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>BioLab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (11)*</td>
<td>57 ± 5</td>
<td>100 ± 12</td>
<td>42 ± 10</td>
</tr>
<tr>
<td>WKY (12)*</td>
<td>51 ± 8</td>
<td>65 ± 8*</td>
<td>14 ± 4*</td>
</tr>
</tbody>
</table>

* Total number of rats perfused.
† P < 0.025 cf. SHR.
‡ P < 0.0125 cf. SHR.

### Table 2 Basal Perfusate Renin Activity*

<table>
<thead>
<tr>
<th>Rats</th>
<th>SHR</th>
<th>WKY</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taconic Farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.62 ± 0.20</td>
<td>(21)</td>
<td>3.00 ± 0.45</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>BioLab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.50 ± 0.20</td>
<td>(11)</td>
<td>5.94 ± 1.11</td>
<td>&lt;0.0025</td>
</tr>
</tbody>
</table>

* Expressed as ng A/1 ml hr⁻¹. Values are expressed as mean ± SEM. Values in parentheses = total number of rats studied.
ence in response was also observed when regression data for SHR and WKY rats from BioLab were compared with their counterparts bred at Taconic Farms (WKY: \( P < 0.0005; \) SHR: \( P < 0.025 \)).

**Kidney Renin Activity (KRA)**

No significant difference could be demonstrated between KRA in the nonperfused right kidneys of Taconic Farms SHR and WKY rats (0.62 ± 0.07 ng A I/ml incubate hr⁻¹ and 0.57 ± 0.07 ng A I/ml incubate hr⁻¹, respectively).

**Discussion**

This investigation has established that the release of renin from the kidney elicited by \( \beta \)-adrenergic agonists such as isoproterenol follows a dose-response pattern similar to that observed with other adrenergically mediated responses. Although this relationship has not been examined previously in the in situ perfused kidney, investigations employing incubated rat kidney slices have demonstrated the catecholamine-induced release of renin to be dose-dependent over a concentration range similar to the one used herein. Other studies using the rat kidney slice technique were unable to show a similar dose-response relationship when higher concentrations of epinephrine, norepinephrine, and isoproterenol were used.

The response of the WKY rats to isoproterenol-evoked renin secretion was consistently greater than that observed in the SHR strains. Although kidney renin activity decreases with age in the SHR⁵,⁶,¹³ and ultimately reaches lower levels than those found in the WKY rats, the marked differences in the regression coefficients of the dose-response curves developed in this study do not appear to be related to kidney renin stores. This conclusion is based on two observations from the present investigation. First, kidney renin activity in the Taconic Farms rats was similar for both WKY and SHR. Second, although kidney renin activity was not determined in BioLab SHR and WKY rats, these data indicate that the highest dose of isoproterenol (5 \( \times 10^{-9} \) M) administered to the BioLab SHR induced a renin secretory response which was greater than the threshold response to isoproterenol (5 \( \times 10^{-11} \) M) obtained in the BioLab WKY rats.

This suggests that the kidney renin content of the BioLab SHR was sufficient to have produced a greater release of renin into the perfusate; consequently, other factors are probably responsible for the attenuated response noted.

Changes in renal perfusion pressure may contribute to the release of renin from the kidney. In Taconic Farms rats, the renal perfusion pressure was greater in the SHR than the WKY rats at both the initiation and termination of the perfusion studies, whereas, in the BioLab rats, a greater perfusion pressure was noted in the SHR only at the termination of the perfusion. Basal perfusate renin activity was significantly higher in the BioLab WKY rats despite perfusion pressures that were initially equal in the two strains obtained from this breeding facility. This finding implies that differences in the renin secretory response observed in the present study are not attributable to alterations in vascular resistance.

The distinctive dose-response relationships observed in the SHR and WKY rats may be attributed to differences

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**TABLE 3 Renin Secretory Response to Isoproterenol**

<table>
<thead>
<tr>
<th>Isoproterenol dosage administered</th>
<th>5 ( \times 10^{-11} )</th>
<th>2.5 ( \times 10^{-10} )</th>
<th>5 ( \times 10^{-10} )</th>
<th>2.5 ( \times 10^{-9} )</th>
<th>5 ( \times 10^{-9} )</th>
<th>2.5 ( \times 10^{-8} )</th>
<th>5 ( \times 10^{-8} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taconic Farms SHR</td>
<td>27 ± 11*</td>
<td>140 ± 40</td>
<td>33 ± 12</td>
<td>241 ± 175</td>
<td>79 ± 51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4)†</td>
<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
<td>(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>67 ± 23</td>
<td>129 ± 64</td>
<td>167 ± 86</td>
<td>558 ± 385</td>
<td>619 ± 221</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
<td>(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioLab SHR</td>
<td>101 (±44)‡</td>
<td>320 (±72)‡</td>
<td>402 (±90)‡</td>
<td>311 ± 9</td>
<td>792 ± 379</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(3)</td>
<td>(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>278 (±222)‡</td>
<td>533 (±113)‡</td>
<td>1133 ± 250</td>
<td>2444 ± 337</td>
<td>3279 ± 189</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(2)</td>
<td>(3)</td>
<td>(3)</td>
<td></td>
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</tr>
</tbody>
</table>

* Area under the curve calculated from perfusate renin activity and expressed as ng A I/ml hr⁻¹ ± SEM.
† Numbers in parentheses = number of rats evaluated at each dose.
°F SEM included for uniformity; note \( n = 2 \).
in the threshold for receptor activation or β adrenoceptor density associated with the adrenergic renin-releasing mechanism. The frequency of sympathetic discharge measured in splanchnic nerves\(^14,15\) or in adrenergic neurons directly innervating the kidney\(^15\) has been shown to be greater in mature SHR than WKY. Whereas an increase in adrenergic activity might be expected to cause elevated basal levels of renin activity in SHR, compared with WKY, the opposite situation has actually been found to exist.\(^12,16\) Although this may be the result of reduced concentrations or turnover of neurotransmitter substance, as has been reported in the SHR for adrenergically innervated tissues other than kidney,\(^10,17,18\) it is possible that genetically unique characteristics of the adrenergic receptor may be contributing to this phenomenon.

An unexpected finding was that dose-response curves constructed from data generated in rats with similar genetic backgrounds but reared in separate breeding facilities were significantly different. Variability in physiological responsiveness within strains has been recognized in SHR and WKY rats during studies of aortic reactivity\(^11\) and sodium excretion.\(^21\) It seems clear, therefore, that genetic selection or environmental influences during substrain breeding can alter strain characteristics that may not be reflected in body weight or systemic blood pressure and that caution must be exercised when analyzing data comparing SHR and WKY rats obtained from more than one breeding facility.

References

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