Hypertension in Experimental Diabetes Mellitus
Renin-Prostaglandin Interaction

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SUMMARY To investigate mechanisms involved in the high incidence of hypertension in diabetes mellitus, the relationship between renin-angiotensin production and renal prostaglandin E\(_2\) synthesis was studied in rats 1 week after diabetes mellitus had been induced by streptozotocin injection. The diabetic rats became hypertensive, although plasma renin activity did not increase despite the plasma volume contraction resulting from polyuria and natriuresis. Subcutaneous insulin injection resulted in a marked increase in plasma renin activity, while more rigid control of diabetes mellitus achieved by constant insulin infusion decreased blood pressure. Cortical renin content and renin release as well as papillary prostaglandin E\(_2\) synthesis in vitro were significantly lower in diabetic rats than in nondiabetic controls. Isoproterenol and prostaglandin E\(_2\) stimulated renin release in controls, while diabetic rats responded only to isoproterenol. Insulin infusion by pump reversed these abnormalities. An additive effect of a maximum dose of isoproterenol (10\(^{-4}\) M) and prostaglandin E\(_2\) (10\(^{-4}\) M) on renin release was observed in nondiabetic controls and in diabetic rats treated with insulin pump, but not in untreated diabetic rats. The results suggest that 1) renal renin release and prostaglandin E\(_2\) synthesis in diabetes mellitus are insulin dependent, 2) inappropriately lower plasma renin activity in diabetes mellitus may be attributed to a diminished renal renin pool and a lack of renin release in response to renal prostaglandin E\(_2\), the synthesis of which is also impaired in diabetes, 3) prostaglandin E\(_2\)-induced renin release may operate independently from isoproterenol-induced renin release, and 4) impaired renal prostaglandin E\(_2\) synthesis may contribute to the development of hypertension in the face of an unchanged prohypertensive renin-angiotensin II system.

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KEY WORDS • sodium excretion • insulin • streptozotocin • hyperglycemia • plasma volume

DIABETES mellitus (DM) is associated with characteristic morphological and functional changes in the kidney. In addition, cardiovascular complications, including hypertension, atherosclerosis, and thrombotic disease, are more prevalent among diabetics than nondiabetics. Increased sensitivity of diabetic platelets to aggregating agents as well as increased diabetic platelet production of thrombox-

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HYPERTENSION IN EXPERIMENTAL DIABETES/Katayama and Lee

streptozotocin-induced diabetes. However, the latter study demonstrated no difference in glomerular 6-keto-prostaglandin F$_{1\alpha}$ and PGE$_2$ synthesis between diabetic and nondiabetic rats.$^4$ On the other hand, plasma renin activity (PRA) is well known to be low in DM, as indicated by hyporeninemic hypoaldosteronism. This suppressed PRA has been reported to be due to volume expansion following hyperglycemia.$^9,^{10}$ impaired renin release,$^{11}$ inadequate conversion of prorenin to renin,$^12$ and diminished catecholamine secretion.$^{13}$

As reported previously, during high renin states such as low sodium diet and renovascular hypertension, increased renal PGE$_2$ synthesis, stimulated by the associated rise in angiotensin II, has an important physiological role in offsetting renin-angiotensin induced renal vasoconstriction and antinatriuresis.$^{14,15}$ Conversely, PGE$_2$, prostaclin, and their precursor arachidonic acid stimulate renin release in humans and experimental animals in vivo and in vitro.$^{16-18}$ Thus, the present study was designed to investigate the alterations in urinary PGE$_2$, excretion and PRA in vivo and renal papillary PGE$_2$ synthesis as well as renin release in vitro in experimental diabetic rats and to correlate the effects of insulin administration with any observed changes in the renin-prostaglandin systems, sodium homeostasis, and blood pressure regulation.

Materials and Methods

Wistar male rats weighing 200 to 250 g (Charles River Breeding Laboratories, Boston, MA, USA) were housed in metabolic cages with free access to tap water and Purina rat chow (normal sodium intake, Ralston Purina Co., St. Louis, MO, USA). To induce diabetes, 65 mg/kg of streptozotocin (Upjohn Co., Kalamazoo, MI, USA) was injected intraperitoneally. The control group received only the vehicle (2.0 ml/kg). The control group revealed no difference from initial values before incubation. All studies were performed after 2 days to determine urinary sodium and potassium concentrations by flame photometry (Instrumentation Laboratory Inc, Boston, MA, USA) and urinary PGE$_2$ excretion by the specific radioimmunoassay described in the following section. After the completion of urinary collection, blood pressure was determined by the tail cuff method. The first pulse appearing on the Grass polygraph recorder (Grass Instrument Co., Quincy, MA, USA) during cuff deflation was assigned as the systolic pressure. After the in vivo studies, the animals were decapitated and truncal blood was collected into a chilled tube containing 5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), and incubated in duplicate for 30 minutes at 37 °C with 95% O$_2$, 5% CO$_2$, in an Eberbach apparatus (A. H. Thomas) at 80 oscillations/min. Slices were homogenized before and after incubation in the medium, and urine and slice homogenates were extracted, and PGE$_2$ was assayed with a highly specific radioimmunoassay (Institute Pasteur, Paris, France) as previously reported.$^{14}$ Results are expressed as nanograms per 24 hours and nanograms per milligram of tissue for urine and slices respectively. For estimation of de novo PGE$_2$ synthesis, initial slice PGE$_2$ content was subtracted from final slice plus medium value and expressed as nanograms per milligram of tissue per 30 minutes. The PGE$_2$ production measured in this fashion reflects de novo synthesis since PGE$_2$ levels after incubation in the absence of oxygen or the presence of indomethacin revealed no difference from initial values before incubation.$^{14}$

In Vitro Studies

Four papillary slices (8–15 mg) were made with a Stadie-Riggs microtome (A. H. Thomas, Philadelphia, PA, USA), placed in 25-ml flasks containing 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), and incubated in duplicate for 30 minutes at 37 °C with 95% O$_2$, 5% CO$_2$, in an Eberbach apparatus (A. H. Thomas) at 80 oscillations/min. Slices were homogenized and diluted in 2 ml of 0.1 M sodium citrate buffer, pH 4.5. Body weight and urine volume were recorded daily. Glycosuria and ketonuria were monitored semiquantitatively with Keto-Diastix reagent strips (Ames Division, Miles Laboratories, Elkhart, IN, USA). Two days after the injection of streptozotocin, plasma glucose levels were determined by the glucose oxidase method using blood samples obtained from the tail vein. Diabetics with plasma glucose levels greater than 300 mg/dl were separated randomly into three groups: 1) untreated diabetics, 2) diabetics treated with 2 units of s.c. lente-insulin (Eli Lilly and Co., Indianapolis, IN, USA) daily for 5 days with one-third in the morning and the remainder in the evening, and 3) diabetics treated with constant infusion of insulin for 5 days by miniosmotic pump (Alza Corp., Palo Alto, CA, USA) implanted subcutaneously in the back during ether anesthesia. Regular insulin was diluted in saline containing 0.7% glutamic acid to block insulin aggregation, and the concentration of insulin in the pump was adjusted to infuse at a rate of 2 U/200 g body weight/24 hr. Infusion of vehicle alone did not show any effect on blood pressure, plasma glucose levels, or PRA.

In Vivo Studies

Five days after the injection of streptozotocin, 24-hour urine samples were collected during the ensuing 2 days to determine urinary sodium and potassium concentrations by flame photometry (Instrumentation Laboratory Inc, Boston, MA, USA) and urinary PGE$_2$ excretion by the specific radioimmunoassay described in the following section. After the completion of urinary collection, blood pressure was determined by the tail cuff method. The first pulse appearing on the Grass polygraph recorder (Grass Instrument Co., Quincy, MA, USA) during cuff deflation was assigned as the systolic pressure. After the in vivo studies, the animals were decapitated and truncal blood was collected into a chilled tube containing 5 ml of ethylenediaminetetraacetic acid for determination of PRA, plasma glucose levels, and hematocrit. The PRA was determined by a specific radioimmunoassay measuring angiotensin I generated at pH 6.0, with an interassay and intraassay variation of 13 and 8% respectively, and expressed as nanograms of angiotensin I per milliliter per hour.$^{19}$

To determine cortical renin content and release in vitro, four to 14 cortical slices (20–40 mg) were prepared from another set of control, untreated diabetic, and insulin-infused diabetic animals as described above. Two cortical slices were immediately homogenized in 2 ml of 0.1 M phosphate buffer with 15 mM EDTA, pH 6.0, with the use of 20 stroke glass homogenizer and diluted with buffer 100 to 250 times for renin assay. Using the incubation technique already described, the remaining slices were incubated in duplicate with or without dl-isoproterenol (10$^{-7}$–10$^{-3}$ M) or PGE$_2$ (10$^{-6}$–10$^{-4}$ M) or both. To achieve these concentrations, isoproterenol (Sigma, St. Louis, MO, USA) was dissolved in Krebs-Ringer bicarbonate buffer containing ascorbic acid to prevent oxidation (final
concentration, 0.1% wt/vol) and PGE₂, originally dissolved in methanol, was diluted in Krebs-Ringer bicarbonate buffer to yield a final methanol concentration of 0.1% v/v, which was determined not to affect renin release or PGE₂ production. All incubation media for initial renin content and basal renin release determination were adjusted to the same concentration of ascorbic acid and methanol. After incubation the medium was diluted 5 to 10 times in 0.1 M phosphate buffer with 15 mM EDTA, pH 6.0. To obtain rabbit renin substrate, rabbits were nephrectomized under general anesthesia (ketamine hydrochloride, 40 mg/kg, and xylazine hydrochloride, 5 mg/kg s.c.) and plasma was withdrawn 48 hours later under the same anesthetic conditions. Fifty microliters of the diluted samples was incubated with 300 μl of rabbit plasma as renin substrate for 1 hour at 37 °C in the presence of EDTA (2.6 mM), dimercaprol (1.6 mM), and 8-hydroxyquinoline (3.6 mM). The reaction was stopped at the end of incubation by the addition of equal amounts of ice-cold phosphate-buffered saline containing 0.1% bovine serum albumin, pH 7.0, and samples were kept frozen at −20 °C until the assay for angiotensin I was performed. Preliminary studies demonstrated that angiotensin I generation in this system was linear, with enzyme concentration ranging from 5 to 200 ng of angiotensin I per milligram of tissue, and renin substrate concentration, estimated by k离ability range from 95 to 200 μl/s per 30 minutes. Renin release in response to isoproterenol or PGE₂ or both also was expressed as nanograms of angiotensin I per milliliter. The recovery of angiotensin I generation in this system was linear, with a correlation coefficient of 0.99. The kinetic assay in the presence of a high concentration of partially purified hog renin (Sigma), was 2650 ng of angiotensin I per milliliter. The recovery of angiotensin I added to the medium during the incubation was 95 ± 3%. Cortical renin content was expressed as nanograms of angiotensin I per milligram of tissue, and renin release as nanograms of angiotensin I per milligram of tissue per 30 minutes. Renin release in response to isoproterenol or PGE₂ or both also was expressed as percentage of basal release without either drug.

**Plasma Volume Determination**

In the other set of control and diabetic animals prepared as described previously, plasma volume was measured by collecting blood samples (100 μl) every 10 minutes for 60 minutes after the injection of ¹²⁵I-labeled bovine serum albumin through a catheter implanted into the right atrium through the jugular vein while the animals were under pentobarbital anesthesia (35 mg/kg i.p.). Radioactivity in plasma was counted and extrapolated to time 0 by the least-squares method. Plasma volume was calculated by dividing total radioactivity injected by the counted radioactivity in 1 ml of plasma at time 0 and expressed as milliliters per 100 g of body weight.

**Statistical Analysis**

All data were expressed as the mean ± se. Statistical significance was determined by the Student's t test for unpaired data.

**Results**

As can be seen in Table 1, the diabetic animals showed a marked rise in plasma glucose levels, urinary volume, sodium excretion, and potassium excretion (p < 0.01). Daily administration of 2 units of insulin significantly decreased plasma glucose concentration when compared with that of untreated diabetics (p < 0.01), which was still higher than that of nondiabetic controls. However, constant infusion of 2 units of insulin by pump resulted in euglycemia. In all the insulin-treated groups urine volume and sodium and potassium excretion, although higher than those of nondiabetic controls, were significantly lower than those in untreated diabetics (p < 0.01).

Blood pressure in diabetic animals was significantly higher than in controls (p < 0.01; Table 2); however, insulin infusion restored the elevated blood pressure to control values. The PRA in untreated diabetic animals (see Table 2) was not different from that in controls; however, there was a marked rise in PRA in insulin-injected diabetics compared with that observed in control and untreated diabetic animals. The statistically insignificant rise in PRA in insulin-infused diabetic animals reached values intermediate between those of controls and insulin-injected diabetics. The hematocrit was slightly elevated in the hyperglycemic animals (p < 0.01).

| TABLE 1. Plasma Glucose Level, Urine Volume, and Urinary Excretion of Sodium and Potassium in Nondiabetic Control and Streptozotocin Diabetic Animals with or Without Insulin Treatment |
|-----------------|-----------------|-----------------|-----------------|
|                 | Nondiabetics    | Untreated       | Insulin (s.c.)  |
|                 |                 |                 | (2 U/24 hr)     | Insulin pump (2 U/200 g/24 hr) |
| Plasma glucose (mg/dl) | 157 ± 7         | 641 ± 17*       | 384 ± 55*±      | 164 ± 48*               |
| UV (ml/24 h)    | 5.5 ± 0.7       | 142.5 ± 13.2*   | 63.8 ± 2.6*±    | 29.8 ± 5.1*+            |
| UKV (mEq/24 h) | 1.1 ± 0.1       | 4.0 ± 0.2*      | 2.1 ± 0.3*+     | 2.2 ± 0.1*+             |
| No. of rats     | 8               | 7               | 8               | 7                       |

Each value represents mean ± se.
See Methods for experimental details.
UV = urinary volume; UKV = urinary excretion of sodium; UKV = urinary excretion of potassium.
*p < 0.01 (vs nondiabetics), f p < 0.01 (vs untreated diabetics)
HYPERTENSION IN EXPERIMENTAL DIABETES/Katayama and Lee

TABLE 2. Blood Pressure, Plasma Renin Activity, and Hematocrit in Nondiabetic Control and Diabetic Animals with or Without Insulin Treatment

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetics</th>
<th>Untreated</th>
<th>Insulin (s.c.) (2 U/24 hr)</th>
<th>Insulin pump (2 U/200 g/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>120 ± 3.5</td>
<td>135 ± 3.1*</td>
<td>115 ± 4.4†</td>
<td></td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>8.6 ± 0.5</td>
<td>8.5 ± 1.5</td>
<td>19.2 ± 4.4*</td>
<td>11.3 ± 2.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.2 ± 0.8</td>
<td>41.7 ± 0.9</td>
<td>42.7 ± 0.6†</td>
<td>39.4 ± 1.5</td>
</tr>
<tr>
<td>No. of rats</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE.

See Methods for experimental details.

ANG I = angiotensin I; PRA = plasma renin activity.
*p < 0.01 (vs nondiabetics), †p < 0.05 (vs nondiabetics), ‡p < 0.01 (vs untreated diabetics).

< 0.05) and decreased to control values in the euglycemic animals treated by insulin infusion. Plasma volume determined in another set of control and untreated diabetics was significantly lower in untreated diabetics (3.73 ± 0.10 ml/100 g) as compared to controls (4.42 ± 0.16 ml/100 g; p < 0.01).

Urinary excretion of PGE₂ is shown in Figure 1. A significant decrease in urinary excretion of PGE₂ was observed in diabetics treated with 2 units of s.c. insulin (p < 0.05), and a significant increase was seen in those treated with 2 units by constant infusion (p < 0.01), as compared to controls and untreated diabetics, which were not significantly different from each other.

Figure 2 shows that papillary PGE₂ slice content in diabetics with and without treatment with subcutaneous injection of insulin was significantly lower than in nondiabetic controls (p < 0.01), although insulin injection partially restored the decreased PGE₂ content found in diabetics. Insulin by constant infusion restored the decreased PGE₂ content to normal values. Although de novo PGE₂ synthesis in untreated diabetics was significantly lower than in controls (p < 0.05), there was a marked decrease in the PGE₂ synthesis rate with injection of 2 units of insulin. In the pump-implanted rats with DM, de novo PGE₂ synthesis was markedly greater than values observed in nondiabetic controls, untreated diabetics, and insulin-injected diabetics. Although blood pressure did not show any significant relation to PRA, a significant negative correlation was obtained between blood pressure and papillary PGE₂ synthesis (p < 0.05; Figure 3).

Basal renin content decreased significantly in diabetic animals as compared to controls, as shown in Table 3 (p < 0.05). Insulin administration by pump partially restored the diminished renin content to control values. Basal renin release, which was also lower in diabetics, returned toward control values with insulin infusion. The percentage of renin released from the
initial renin content was significantly lower in diabetics as compared to controls \((p < 0.05)\), which was a reflection of a greater decrease in basal diabetic renin release. Insulin infusion restored the percentage of renin released to normal control values. Figure 4 shows renin release in vitro in response to direct addition of the \(\beta\)-adrenergic stimulator isoproterenol and PGE\(_2\) to the medium. Isoproterenol resulted in a significant increase in the percent of renin released \((p < 0.01)\), which did not differ among controls, untreated diabetics, and insulin-infused diabetics. However, the absolute value of maximal renin release with isoproterenol was lower in untreated diabetics \((4.82 \pm 0.54 \text{ ng of angiotensin I per milligram per 30 minutes})\) than in controls \((8.91 \pm 0.80 \text{ ng of angiotensin I per milligram per 30 minutes})\). In contrast, PGE\(_2\) did not stimulate renin release even in high concentrations in untreated diabetic animals as compared to controls and animals receiving insulin by pump infusion.

Figure 5 shows an additive effect of isoproterenol and PGE\(_2\), on renin release in vitro in normal and insulin-infused diabetic animals but the absence of such an effect in untreated diabetic animals.

Discussion

The present study demonstrates that diabetic rats are hypertensive as early as 1 week after the induction of DM by streptozotocin administration and that insulin infusion reduces the elevation in blood pressure. These findings confirm recent studies by Bunag et al.\(^{20,21}\) In these studies, diabetic rats in which hypertension was established 2 or more weeks after the onset of DM were characterized by narrowed pulse pressure, bradycardia with increased reflex responses to norepinephrine, and reduced pressor responses to hypothalamic stimulation and vasopressin. In addition to these abnormalities, the present studies demonstrate that PRA was not stimulated in untreated diabetic rats in spite of a marked sodium diuresis, which is consistent with

### Table 3. Cortical Renin Content, Basal Renin Release, and Percent of Renin Release in Nondiabetic Control and Diabetic Animals with or Without Insulin Infusion

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetics</th>
<th>Untreated</th>
<th>Insulin pump ((2 \text{ U/200 g/24 hr}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>137 ± 6</td>
<td>504 ± 28*</td>
<td>86 ± 11†</td>
</tr>
<tr>
<td>Renal renin content (ng ANG I/mg)</td>
<td>232 ± 14.9</td>
<td>187 ± 15.8†</td>
<td>202 ± 28.1</td>
</tr>
<tr>
<td>Basal renin release (ng ANG I/mg/30 min)</td>
<td>4.96 ± 0.33</td>
<td>3.01 ± 0.33*</td>
<td>3.88 ± 0.46</td>
</tr>
<tr>
<td>Renin release (%)</td>
<td>2.28 ± 0.20</td>
<td>1.71 ± 0.22†</td>
<td>2.06 ± 0.28</td>
</tr>
<tr>
<td>No. of rats</td>
<td>9</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE.

See Methods for experimental details.

ANG I = angiotensin I

*\(p < 0.01\) (vs nondiabetics), †\(p < 0.05\) (vs nondiabetics), ‡\(p < 0.01\) (vs untreated diabetics).
Thus, the intermediate step(s) from β-adrenergic stimulation to the ultimate release of renin itself may not be impaired in DM based on its normally preserved renin release, which involves independent mechanisms for renin release. If, as some believe, PGE₂-mediated renin release has different mechanisms that are independent from β-adrenergic renin release, even if cyclic adenosine monophosphate is the final mediator in both of them (Figure 6). Thus, impaired renin release in untreated DM does not appear to be the result of β-adrenergic insensitivity but may be a reflection of impaired renin synthesis in diabetic animals receiving s.c. insulin cannot be explained by this hypothesis, and a direct effect of insulin on renin synthesis in diabetic rats has been shown to be derived from a storage pool of previously synthesized, but not newly synthesized, renin, which again suggests that DM leads to a diminished renal renin synthesis resulting in a decreased size of renin pool that responds normally to β-adrenergic stimulation.

The β-adrenergic agonist isoproterenol is believed to directly stimulate juxtaglomerular β-adrenergic receptors, which results in renin release. The prostaglandin synthesis inhibitor indomethacin has been reported to inhibit not only renin release stimulated by upright posture, furosemide, and hemorrhage but also isoproterenol-induced renin release. Recently, Campbell et al. reported that dibutyril cyclic adenosine monophosphate–induced renin release was also blocked by indomethacin. Despite these observations suggesting a prostaglandin-β-adrenergic link in the mechanism of renin release, recent data do not support such an interaction of prostaglandins with the β-adrenergic system. In the present studies, maximal concentrations of PGE₂ and isoproterenol showed additive effects on renin release in normal and insulin-treated diabetic rat renal cortical slices, which again suggests an action of PGE₂ independent of that of β-adrenergic mechanisms. This hypothesis was also supported by the finding that no such additive effect was observed in untreated diabetic rats, which displayed impaired renin release in response to PGE₂ but normal responses to isoproterenol. If, as some believe, PGE₂ is involved in the final pathway by which isoproterenol releases renin at a step beyond cyclic adenosine monophosphate formation, the response of renin to both these compounds would more likely be impaired to the same extent in diabetics. Since this does not appear to be the case, the present studies indicate that PGE₂-mediated renin release has different mechanisms that are independent from β-adrenergic renin release, even if cyclic adenosine monophosphate is the final mediator in both of them (Figure 6). Thus, impaired renin release in untreated DM does not appear to be the result of β-adrenergic insensitivity but may be a reflection of a decreased basal renin content that is sensitive to an insulin-mediated action on renin release by PGE₂. The high PRA and low PGE₂ synthesis and excretion in animals receiving s.c. insulin cannot be explained by this hypothesis, and a direct effect of insulin on renin synthesis in diabetic animals will be lower in diabetes than in controls. Isoproterenol-stimulated renin release has been shown to be derived from a storage pool of previously synthesized, but not newly synthesized, renin, which again suggests that DM leads to a diminished renal renin synthesis resulting in a decreased size of renin pool that responds normally to β-adrenergic stimulation.

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The β-adrenergic agonist isoproterenol is believed to directly stimulate juxtaglomerular β-adrenergic receptors, which results in renin release. The prostaglandin synthesis inhibitor indomethacin has been reported to inhibit not only renin release stimulated by upright posture, furosemide, and hemorrhage but also isoproterenol-induced renin release. Recently, Campbell et al. reported that dibutyril cyclic adenosine monophosphate–induced renin release was also blocked by indomethacin. Despite these observations suggesting a prostaglandin-β-adrenergic link in the mechanism of renin release, recent data do not support such an interaction of prostaglandins with the β-adrenergic system. In the present studies, maximal concentrations of PGE₂ and isoproterenol showed additive effects on renin release in normal and insulin-treated diabetic rat renal cortical slices, which again suggests an action of PGE₂ independent of that of β-adrenergic mechanisms. This hypothesis was also supported by the finding that no such additive effect was observed in untreated diabetic rats, which displayed impaired renin release in response to PGE₂ but normal responses to isoproterenol. If, as some believe, PGE₂ is involved in the final pathway by which isoproterenol releases renin at a step beyond cyclic adenosine monophosphate formation, the response of renin to both these compounds would more likely be impaired to the same extent in diabetics. Since this does not appear to be the case, the present studies indicate that PGE₂-mediated renin release has different mechanisms that are independent from β-adrenergic renin release, even if cyclic adenosine monophosphate is the final mediator in both of them (Figure 6). Thus, impaired renin release in untreated DM does not appear to be the result of β-adrenergic insensitivity but may be a reflection of a decreased basal renin content that is sensitive to an insulin-mediated action on renin release by PGE₂. The high PRA and low PGE₂ synthesis and excretion in animals receiving s.c. insulin cannot be explained by this hypothesis, and a direct effect of insulin on renin synthesis in diabetic animals will be lower in diabetes than in controls. Isoproterenol-stimulated renin release has been shown to be derived from a storage pool of previously synthesized, but not newly synthesized, renin, which again suggests that DM leads to a diminished renal renin synthesis resulting in a decreased size of renin pool that responds normally to β-adrenergic stimulation.
release in these hyperglycemic animals cannot be excluded. Whether exogenous PGE\(_2\) has a different action from endogenous PGE\(_2\)-mediated renin secretion in juxtaglomerular cells or whether exogenous PGE\(_2\) modulates cyclic adenosine monophosphate, either by adenylyl cyclase activation or cyclic adenosine monophosphate degradation, remains to be clarified.

In addition to the abnormalities already discussed, we found that papillary PGE\(_2\) biosynthesis in vitro was lower in untreated diabetic animals and in the diabetic group treated with 2 units of s.c. insulin injection, although the untreated diabetic rats had a normal urinary PGE\(_2\) excretion. This phenomenon might be explained, based on intrarenal washout effect, by a marked polyuria induced by DM. In fact, rats with DM showed a marked decrease in initial PGE\(_2\) content that was partially restored as polyuria was decreased by s.c. insulin injection at 2 U/24 hr despite a continuously lower PGE\(_2\) synthesis rate. Interestingly, a constant infusion of 2 units of insulin by pump in diabetic animals resulted in a marked increase in renal PGE\(_2\) synthesis exceeding that of controls and untreated diabetics and led to complete normalization of PGE\(_2\) levels in vitro and urinary PGE\(_2\) excretion in vivo, which is in accord with the previously proposed hypothesis for an action of insulin on renin release mediated by PGE\(_2\). It must be recognized, however, that streptozotocin has effects other than hypoglycemia (e.g., oxidant tone) that may have an effect on prostaglandin synthesis.

In summary, these results suggest that any dependency of renal PGE\(_2\) on the renin-angiotensin axis as well as renin release on PGE\(_2\) is conditioned by a permissive action of the normal presence of insulin, which modulates interactions between renin and PGE\(_2\). The difference in efficiency of insulin to restore or increase PGE\(_2\) levels or synthesis may be attributed to the method of administration, since subcutaneous injection results in peaks and valleys of insulin plasma concentration, while constant infusion results in a relatively steady state of insulin release. Whether insulin per se stimulates PGE\(_2\) synthesis directly or indirectly through changes in the renal arachidonic cascade remains to be clarified. In addition, although urinary PGE\(_2\) excretion in vivo appeared to be a function of synthesis and urine volume, we cannot exclude the possibility that insulin affects PGE\(_2\) degradation. Physiological implications of a decreased renal PGE\(_2\) production and responsiveness of renin to PGE\(_2\) in the alterations of renal hemodynamics, sodium handling, and blood pressure regulation in DM are also unknown. However, a decrease in vasodilatory PGE\(_2\) resulting in a relative preponderance of vasoconstricting renin-angiotensin system, even if inappropriately lower in relation to volume contraction, might explain in part certain of the pathological phenomena in DM including the mild hypertension in streptozotocin-induced diabetic rats observed in this study and in others, and the reduction in glomerular filtration rate and renal plasma flow in human and prolonged experimental DM.

Finally, the marked natriuresis and polyuria observed in untreated diabetic humans and animals may be attributed not only to an osmotic effect of glycosuria but to a deficiency of an appropriate insulin-mediated rise in the renin-angiotensin-aldosterone axis, which bears a pathophysiological resemblance to renal events associated with adrenal insufficiency in the sense that in both conditions an inappropriately low aldosterone level may underlie the renal polyuria and sodium loss.

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