Decreased Angiotensin II-Stimulated Aldosterone Production, but Normal Inositol Phosphate Response in Adrenal Glomerulosa Cells from Streptozotocin-Induced Diabetic Rats: Role of Insulin†

Seong Yeon Kim1, Yeon Ah Sung, Bo Youn Cho, Hong Kyu Lee, Chang-Soon Koh and Hun Ki Min

Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-799, Korea

Abstract = Streptozotocin(STZ)-induced diabetic rats develop hyporeninemic hypoaldosteronism during the progression of diabetes mellitus. However, the nature and mechanism of aldosterone deficiency in diabetic rats still remain unclear and acute effects of insulin on aldosterone production in-vitro are not known. We evaluated the responses of aldosterone production to angiotensin II (AII), potassium (K+), ACTH and insulin in adrenal glomerulosa cells prepared from STZ-induced diabetic rats with and without insulin treatment 2 weeks after diabetes induction. We also measured inositol phosphate(IP) levels in AII-stimulated glomerulosa cells labeled with [3H]myoinositol using standardized anion exchange chromatography. Plasma renin activity and aldosterone level were not different among control rats, untreated and insulin-treated diabetic rats. Basal aldosterone production was similar in cells from the three groups. Cells from untreated diabetic rats showed a significant decrease in the maximal AII (10^-8M)-stimulated aldosterone production and a tendency to be low in the maximal K+(8.7 mM)-stimulated aldosterone production, compared with control rats (3.2±2.2 vs 7.7±2.4, P < 0.05 and 4.8±1.8 vs 8.0±3.2 ng/10^5 cells/hr, 0.05 < P < 0.1, respectively). In contrast, there were no differences in AII- and K+ stimulated aldosterone production between control and insulin-treated diabetic rats. ACTH (10^-8M), however, caused a similar effect on aldosterone production and insulin (1 mU/ml for 1 hour) did not alter either basal or agonists-stimulated aldosterone production in cells from the three groups. All (10^-8M)-induced IP formation among the three groups was similar and did not change with the addition of insulin (1 mU/ml). These results indicate that reduced response to AII in the early phase of STZ-induced diabetes in rats may be due to the zona glomerulosa dysfunction secondary to chronic lack of insulin and the main defect responsible for altered AII effects may be located at some step(s) mediating AII action downstream from IP formation.

Key Words: Aldosterone, Angiotensin II, Inositol phosphate, Streptozotocin, Diabetic rats

† Author for correspondence: Tel. (02) 760-3216, Fax. (02) 762-9662

Received in April 1994, and in final form June 1994.

† This study was supported by a grant No. 04-91-002 from Seoul National University Hospital Research Fund
INTRODUCTION

It has been recognized that diabetic rats induced by an injection of STZ develop hyporeninemic hypoadosteronism during the progression of diabetes and insulin treatment can reverse the STZ-evoked effects (Hayashi et al. 1964; Pratt et al. 1985; Kigoshi et al. 1986; Rebuffat et al. 1988 & 1990; Azukizawa et al. 1991). However, there is still some controversy about the nature of the hypoadosteronism in diabetic rats. Several studies have revealed that the in vivo responses of aldosterone production to physiologic agonists such as All, K⁺ and ACTH, are reduced in diabetic rats (Hayashi et al. 1984; Rebuffat et al. 1988) On the other hand, other investigators could not demonstrate the same findings in vitro. Kigoshi et al. (1986) and Azukizawa et al. (1991) found selective unresponsiveness of aldosterone production to All but not to ACTH in adrenal glomerulosa cells from STZ-induced diabetic rats with hypoadosteronism. Nadler et al. (1990) reported that whereas All-induced aldosterone stimulation was markedly reduced, K⁺ and ACTH-mediated effects on aldosterone production were enhanced in diabetic rats.

Little information exists on the exact mechanisms involved in altered All response in diabetic rats. One study has shown that the formation of 12-hydroxyeicosatetraenoic acid, which is a 12-lipoxygenase product and one possible mediator of All-induced aldosterone synthesis, was attenuated in diabetic rats (Nadler et al. 1990). Other important intracellular mediators of All action, however, were not investigated in that study. Recently Azukizawa et al. (1991) observed the number and affinity of All receptors were normal in the diabetic rats with hypoadosteronism and speculated that the main defect responsible for the hypoadosteronism may be located at some steps downstream from All binding.

There is strong evidence that insulin directly or indirectly affects steroidogenesis in adrenal zona glomerulosa. Insulin could act by affecting metabolic conditions within the adrenal or by a growth promoting or adrenotrophic action (Straus 1981). In this regard, steroidogenesis from ovarian or adrenal cortical cells in vitro clearly increases with the addition of insulin (Veldhuis et al. 1983; Penhoat et al. 1988). Receptors for insulin have now been identified in the adrenal (Marques et al. 1982; Penhoat et al. 1988; Pillon et al. 1988) including zona glomerulosa (Bergerson et al. 1980). Furthermore, during a short-term infusion of insulin All has also been known to augment aldosterone secretion in dogs (Rocchini et al. 1990) and in humans (Viehapper et al. 1983). These studies, however, have been performed to elucidate long-term or in-vivo effects of insulin on steroidogenesis including aldosterone production and there have been few studies to investigate acute effects of insulin on aldosterone production in-vitro.

This study was undertaken to investigate the nature and mechanism of alteration in aldosterone production in STZ-induced diabetic rats. In addition, we assessed aldosterone production during a short-term incubation of adrenal zona glomerulosa cells with insulin. In an attempt to clarify the mechanism whereby All-induced aldosterone production is reduced in diabetic rats, we examined inositol phosphate formation via phosphatidylinositol turnover which is regarded as the main signal transduction mechanism utilized by All in stimulating aldosterone production (Hunyady et al. 1982; Woodcock et al. 1988) and also determined the acute effect of insulin on All-induced inositol phosphate formation.

MATERIALS AND METHODS

STZ and crude collagenase (type I) were obtained from Sigma Chemical Co. (St. Louis, MO); synthetic All (human) from Bachem (Switzerland), synthetic ACTH(1-24) from Organon (Nederland), insulin from GlucoBRL (Grand Island, NY) and [³H]-myoinositol from New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma Chemi-
Sprague-Dawley male rats weighing 200 to 250g were fed on a commercial chow and tap water ad libitum throughout the experiment. 60 rats were randomly divided into three groups. Two groups were given a single intraperitoneal injection of streptozotocin (60mg/kg, dissolved in 0.5ml, pH 4, citrate buffer). Diabetes induction was evidenced by conspicuous hyperglycemia and/or severe glycosuria 2-3 days after an injection of streptozotocin. A subset of diabetic rats was treated with intermediate acting insulin (1 U human insulin, Novo Nordisk, Denmark)/kg administered s. c. daily at 9:00 A.M.) and the insulin dose were slightly modified according to rat weights and the previous day’s blood glucose levels. The non-diabetic control and the non-insulin-treated diabetic groups received daily injection of saline. Since Rebuffat et al. (1990) demonstrated STZ-induced diabetic rats already develop hyporeninemic hypoaldosteronism within one week after diabetes induction, we used diabetic rats at 2 weeks after diabetes induction. These animals were decapitated, the adrenal were excised and the trunk blood for measurements of plasma hormonal and biochemical parameters were collected in chilled tubes containing EDTA and promptly centrifuged. Plasma was separated, frozen and stored at -70°C.

The excised adrenals were bisected and decapsulated. The adrenal glomerulosa cells were prepared by the collagenase dispersion technique of Braley et al. (1977) with slight modification. In brief, the capsule were incubated in collagenase buffer at 37°C for 50 minutes in a Dubnoff metabolic shaking incubator. The collagenase buffer consisted of 3.5mg/ml collagenase in Krebs-Ringer-HEPES buffer (KRH) containing 0.1% bovine serum albumin (BSA) and 10 mM glucose with the potassium concentration adjusted to 3.5 mM (0.1% BSA-KRH). KRH buffer contained 135mM NaCl, 2.5mM KCl, 1mM KH₂PO₄, 1mM MgSO₄, 1.25mM CaCl₂, 10mM glucose and 20 mM HEPES (pH 7.4). After incubation, the cells were dispersed mechanically, filtered through nylon mesh (pore size, 200 μm) and centrifuged at 1000 r. p. m. for 10 minutes. The supernatant was discarded, and the cell pellet was washed twice with 0.1% BSA-KRH. The washed cell pellet was resuspended in 0.1% BSA-KRH and preincubated at 37°C for 60 minutes in a Dubnoff metabolic shaking incubator before the main experiment. Cells were counted in a hemocytometer and cell viability was assessed by trypan blue exclusion. Over 90% of cells excluded trypan blue and fasciculated cells were contaminated in less than 10% of glomerulosa cells in this cell preparation.

To determine the responses of aldosterone production to All, K⁺, ACTH and insulin, the glomerulosa cells (1x 10⁵ cells per tube) were incubated in duplicate or triplicate at 37°C for 60 minutes in a Dubnoff metabolic shaking incubator in a final volume of 0.5ml 0.1% BSA-KRH with or without 10⁻⁸ M All, 8.7mM K⁺, 10⁻⁶M ACTH or 1μM/ml insulin. In this experiment, 10⁻⁸ M All, 8.7mM K⁺ and 10⁻⁶ M ACTH were used for acute stimulation of the glomerulosa cells. These concentrations of agonists were known to maximally stimulate aldosterone production by the glomerulosa cells of rats (Braley et al. 1986). Aldosterone production did not change when adrenal glomerulosa cells were incubated with various concentrations of insulin for 1 hour in the present study (data not shown). We used 1μM/ml insulin in this experiment, as suggested by Nadler et al. (1990). After 1-hour incubation the cells were sedimented by centrifugation and the supernatants were stored at -20°C until assayed for aldosterone. This experiment was repeated 5 times.

Plasma renin activity and aldosterone level (or aldosterone in the media) were measured directly by radioimmunoassy using commercial kits from Sorin Biomedical (Italy) and Diagnostic Products Corporation (California, U.S.A) respectively. Plasma sodium and potassium were determined by flame photometry. Plasma glucose was determined by glucose oxidase method using glucose analyzer from Yellow Spring Instrument (U.S.A).
Measurement of inositol phosphates

Inositol phosphates were measured using the method of Woodcock et al. (1988) with slight modification. This method is described briefly below.

After preparation of adrenal glomerulosa cells, cells were resuspended in HEPES-buffered Ham's F-12 medium (pH 7.4) containing 24mM NaHCO₃, 0.1% BSA and 100 U/ml each of penicillin and streptomycin. [³H]myoinositol (10 uCi/ml; 0.55uM) was added, and cells (1.5–2.0 x 10⁶/ml/group) were incubated in organ tissue culture dish (60 x 15 mm, Falcon) for 18:20 hours at 37°C to label inositol phospholipid. Labeled cells were washed twice with KRH. [³H]myoinositol-labeled cells (5-7x10⁹) were incubated in plastic tubes in 0.3 ml KRH containing 10 mM LiCl and 10 minutes later 10⁻⁵ All with and without 1mU/ml insulin was added and incubation continued for the period specified. Incubation was terminated by adding 0.3 ml 10% HClO₄. Samples were allowed to stand on ice for 10 min and were then centrifuged to precipitate protein and phospholipid. Supernatants were collected and neutralized with 0.6 ml of 1M KOH (pH 7.0). KCIO₄ was then precipitated at 0°C for 15 min on ice and removed by brief centrifugation. Samples of neutralized extracts were diluted to 5 ml with distilled water. Diluted samples were applied directly to 1.5 ml column of AG 1 x 8 (200-400 mesh,formate form)anion monophosphate(IP₃) were eluted with 12ml of 0.2 M ammonium formate-0.1 M formic acid, inositol bisphosphate (IP₃) with 12ml of 0.4 M ammonium formate-0.1 M formic acid and inositol triphosphate(IP₃) with 12ml of 1 M ammonium formate-0.1 M formic acid. The column was calibrated prior to the experiment, with tritiated standards of IP₃ (New England Nuclear). 4 ml column fractions were collected sequentially and each 4-ml elute was then partially evaporated by vaccum centrifugation. Each 3 ml residue was mixed with 1 ml of scintillation fluid (Aquasol-II, New England Nuclear) and counted in a Beckman scintillation counter. The present experiments were repeated on five occasions.

Data analysis

Data are expressed as mean ± S. D. Statistical analysis was done, using an SAS statistical package. Group means were compared by unpaired Student's t test or one-way analysis of variance with Scheffe's method. P-value less than 0.05 was regarded as significant.

RESULTS

Table 1 shows the effects of diabetes with and without insulin treatment on body weight, blood glucose, electrolytes and hormone levels. Body weight was significantly lower and plasma glucose higher in untreated diabetic rats than in control rats. Insulin treatment of diabetic rats resulted in marked changes in body weights.

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>Diabetic rats</th>
<th>Diabetic rats treated with insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=27)</td>
<td>(n=24)</td>
<td>(n=27)</td>
</tr>
<tr>
<td>Body weight(g)</td>
<td>260±34</td>
<td>167±29*,**</td>
<td>217±39*</td>
</tr>
<tr>
<td>Plasma glucose(mg/dl)</td>
<td>121±10</td>
<td>454±57*,**</td>
<td>181±70*</td>
</tr>
<tr>
<td>Plasma aldosterone(pg/ml)</td>
<td>604±284</td>
<td>688±219</td>
<td>586±302</td>
</tr>
<tr>
<td>Plasma renin activity(ng/ml/hour)</td>
<td>1.85±0.86</td>
<td>1.79±1.26</td>
<td>1.84±1.03</td>
</tr>
<tr>
<td>Plasma Na(mEq/L)</td>
<td>163±3</td>
<td>157±4*</td>
<td>161±2</td>
</tr>
<tr>
<td>Plasma K(mEq/L)</td>
<td>7.9±0.6</td>
<td>7.8±1.2</td>
<td>8.0±1.1</td>
</tr>
</tbody>
</table>

Values given are Mean ± S.D. Number of rats in parenthesis.

*: P<0.05 vs. control rats, **: P<0.05 vs. insulin-treated diabetic rats

#: Electrolytes were measured in 10 rats.
and plasma glucose, but insulin-treated diabetic rats still had significantly lower weights and higher plasma glucose than control rats. Plasma sodium was decreased in untreated diabetic rats, compared with control and insulin-treated diabetic rats. (P<0.05) On the other hand, there were no significant differences among the three groups in the plasma potassium, plasma renin activity and plasma aldosterone. Basal and 1mU/ml insulin-stimulated aldosterone production were similar in the cells from the three groups, as shown in Fig. 1. The responses of aldosterone production to 10⁻⁸ M All alone or All plus 1mU/ml insulin in the cells from control rats, untreated and insulin-treated diabetic rats are shown in Fig. 1. The maximal response to All was significantly lower in the cells from untreated diabetic rats than in the control rats (3.2±2.2 vs 7.7±2.4 ng/10⁶ cells/ hr, P<0.05). This effect of the diabetic state on All-stimulated aldosterone production was not observed with insulin treatment (6.9±4.5 ng/10⁶ cells/ hr in insulin-treated diabetic rats). Although the cells from untreated diabetic rats had a lower response of aldosterone production to 10⁻⁸ M All plus 1 mU/ml insulin than those of control and insulin-treated diabetic rats, this difference was not statistically significant (P>0.05). In addition, 1-hour incubation of the cells from the three groups with insulin (1mU/ml) resulted in no changes of All-stimulated aldosterone production.

The responses of aldosterone production to K⁺ (8.7mM) alone or K⁺ (8.7mM) plus insulin (1mU/ml) tended to be lower in the cells from diabetic rats than in the cells from control and insulin-treated diabetic rats (4.8±1.8 vs 8.0±3.2, 8.3±3.0 ng/10⁶ cells/ hr and 4.7±1.8 vs 7.8±2.7, 8.2±2.8 ng/10⁶ cells/ hr, respectively), as shown in Fig. 2. The difference, however, was not statistically significant (P>0.05). Insulin (1mU/ml) did not affect the maximal aldosterone response to K⁺ in the cells from the three groups.

The responses of aldosterone production to 10⁻⁸ M ACTH alone or 10⁻⁸ M ACTH plus 1mU/ml insulin were not different in the cells from control rats, untreated and insulin-treated diabetic rats (P>0.05), as shown in Fig. 3. ACTH-stimulated aldosterone production in the cells from the three groups did not alter with the addition of 1mU/ml insulin.
Fig. 3. Responses of aldosterone production to ACTH (10⁻⁶M) alone and ACTH (10⁻⁸M) plus insulin (1mU/ml) in adrenal zona glomerulosa cells from control, untreated and insulin-treated diabetic rats (n=5). Bar denotes S.D.. There were no significant differences in aldosterone production among the three groups.

Table 2. Inositol phosphate response to angiotensin II and insulin

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>Diabetic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>All</td>
</tr>
<tr>
<td><strong>IP₁</strong></td>
<td>2075</td>
<td>5253</td>
</tr>
<tr>
<td>±1113</td>
<td>±2870</td>
<td>±2740</td>
</tr>
<tr>
<td><strong>IP₂</strong></td>
<td>551</td>
<td>1507</td>
</tr>
<tr>
<td>±238</td>
<td>±809</td>
<td>±738</td>
</tr>
<tr>
<td><strong>IP₃</strong></td>
<td>296</td>
<td>631</td>
</tr>
<tr>
<td>±118</td>
<td>±241</td>
<td>±257</td>
</tr>
</tbody>
</table>

Values given are mean ± S.D. Results shown are inositol phosphate accumulation in counts per min (mean ± S.D.) x 10⁶ cells at 30 seconds. The experiments was performed five times, using 1.5-2.0 x 10⁶ cells/ml/group. All: 10⁻⁶ M angiotensin II, Ins: 1 mU/ml insulin IP₁: inositol monophosphate, IP₂: inositol bisphosphate, IP₃: inositol triphosphate. There were no differences in inositol phosphate response among the three groups.

adrenal glomerulosa cells with All (10⁻⁶M) and LiCl (10mM) resulted in rapid increases in the levels of IP₁, IP₂, and IP₃. As shown in Fig. 4, maximal increases in IP₂ and IP₃ were detected as early as 15 sec whereas IP₁ increases continuously during 20 min after the addition of 10⁻⁶M All. Because IP₁, IP₂, and IP₃ remained elevated significantly above basal level 30 sec after the addition of All, these inositol phosphates were measured at 30 sec after the addition of All and insulin. As shown in table 2, basal IP₁, IP₂, and IP₃ levels were not different among control, untreated and insulin-treated diabetic rats and incubation with 10⁻⁶M All resulted in similar increases in these inositol phosphates levels. All- induced inositol phosphate formation in the three groups did not alter with the addition of 1mU/ml insulin as well.
DISCUSSION

In the present study, we found the attenuated in-vitro response of aldosterone production to All but not to ACTH in the adrenal glomerulosa cells from the STZ-induced diabetic rats 2 weeks after diabetes induction in spite of normal plasma renin activity, aldosterone and K levels, and also observed that insulin treatment completely reversed these effects of experimental diabetes, which therefore cannot be reasonably ascribed to a direct toxic effect of STZ.

Hyporeninemic hypoaldosteronism has previously been reported to occur in the STZ-induced diabetic rats (Hayashi et al. 1984; Pratt et al. 1985; Kigoshi et al. 1986; Rebuffat et al. 1988 & 1990; Azukizawa et al. 1991) This is manifested by low levels of plasma renin activity and aldosterone level with normal glucocorticoid function: Rebuffat et al. (1988, 1990) reported that diabetic rats had low levels of plasma renin activity and plasma aldosterone 2 weeks after injection of STZ, and subsequently found that basal and All-stimulated plasma aldosterone levels displayed a significant decrease as early as 7 days after diabetes induction and were linearly related to the duration of experimental diabetes. The difference between our findings of normoreninemic normoaldosteronemia and the previous reports (Hayashi et al. 1984; Pratt et al. 1985; Kigoshi et al. 1986; Azukizawa et al. 1991) including those of Rebuffat et al. (1988,1990) cannot be easily explained. This may be related to the difference in the severity of diabetes since the mean plasma glucose was about 450mg/dl in the present study and more than 550mg/dl in their studies. In contrast Wilkes(1987) observed normal plasma renin activity and elevated rather than normal or decreased plasma aldosterone in the STZ-induced diabetic rats 7-80 days after diabetic induction. Further studies are needed to elucidate the changes of the renin-angiotensin-aldosterone system in diabetic rats during the progression of diabetes mellitus.

In agreement with the previous reports (Kigoshi et al. 1986; Azukizawa et al. 1991), adrenal glomerulosa cells from diabetic rats without insulin treatment showed the attenuated response to All but not to ACTH in the present study, suggesting that the diabetic rats may have selective impairment of glomerulosa cells to All because the mechanism of actions of All and ACTH on aldosterone production is different (Braley et al. 1986). Two possibilities have been considered to explain such aldosterone secretory response. Chronic insulin deficiency may play a role in the attenuated aldosterone response in STZ-induced diabetic rats. Several findings support this view: 1) A positive effect of insulin on the growth of several cell types has been observed in vitro (Straus 1981), and specific receptors for insulin and insulin-like growth factor I has been demonstrated in adrenocortical cells (Bergeron et al. 1980; Marques et al. 1982; Penhoat et al. 1988; Pillion et al. 1988) 2) Insulin has been found to raise the activity of steroid-synthesizing enzymes and consequently stimulate steroid secretion by adrenocortical cells cultured in vitro (Penhoat et al. 1988). 3) In the present study, insulin treatment completely reversed the STZ-evoked effects at 2 weeks, as previously reported (Pratt et al. 1985; Rebuffat et al. 1988). Several reports have suggested that hyporeninemia and its attendant All deficiency may be responsible for selective impairment of glomerulosa cells to All (Kigoshi et al. 1986; Rebuffat et al. 1988). Since All has been known to be involved in the maintenance and stimulation of rat zona glomerulosa growth and secretory capacity (Nussdorfer 1986), a lack of prolonged exposure in vivo of adrenal tissues to All could be responsible for this selective impairment. The results of normal plasma renin activity and its resulting possibly normal plasma All in the present study, however, do not exclude the possibility that chronic All deficiency may cause the decreased aldosterone responses in the diabetic rats. Accumulating evidence shows that All produced locally from adrenal tissue is important in aldosterone pro-
duction and the concentration of local Ali is quite different from that of blood Ali (Parkinson et al. 1984; Kifor et al. 1991). However, our results of normal plasma renin activity and altered Ali response suggest that chronic lack of insulin may play a more important role than chronic deficiency of Ali in the attenuated aldosterone production in the STZ-induced diabetic rats during the early phase of diabetes.

Of interest, the cells from diabetic rats had a tendency to be low in the maximal K⁺-stimulated aldosterone production. This result is consistent with the report by Kigoshi et al. (Kigoshi et al. 1986). They observed that the STZ-induced diabetic rats develop hyporeninemic hypaldosteronism as well as altered K⁺ response and speculated that such a subnormal response of aldosterone production to K⁺ in the diabetic rats may be due to chronic deficiency of Ali since there is strong evidence that an Ali-K⁺ interdependency is important in their separate stimulatory potentials (Foster et al. 1979; Parkinson et al. 1984).

The in vivo studies have shown that diabetic rats have a reduced response of aldosterone production to ACTH as well as Ali or K⁺ (Hayashi et al. 1984; Rebuffat et al. 1988). The in vitro studies (Kigoshi et al. 1986; Azukizawa et al. 1991) including the present study demonstrated that diabetic rats have an altered glomerulosa cell response to Ali or K⁺ but not to ACTH. The difference between the in vivo and in vitro studies cannot be explained.

In the present study a short-term in-vitro incubation of the glomerulosa cells from control rats, untreated and insulin-treated diabetic rats with insulin caused no changes in both basal and agonists-stimulated aldosterone production, suggesting insulin does not directly affect aldosterone production in-vitro during a short-term period. These results are inconsistent with those of Nadler et al. (1990) who described insulin (1μU/ml) potentiated Ali-induced aldosterone synthesis in the STZ-induced diabetic rats. The reason for this discrepancy is unclear. Rocchini et al. (1990) and Vierhapper et al. (1983) demonstrated in dogs and healthy men that during euglycemic hyperinsulinemia (hyperinsulinemia induced by insulin infusion for 2 hours) Ali infusion augments aldosterone secretion. This difference may be related to the differences of the experiment condition since we used rats and performed the in vitro experiment, and they used dogs and healthy men and performed the in vivo experiments. Contrary to the in vitro experiment, other factors in addition to insulin per se, can affect the response of aldosterone production in the in vivo experiment.

The mechanism(s) responsible for the attenuated response of aldosterone production to Ali in the STZ-induced diabetic rats is unclear. Azukizawa et al. (1991) observed that the adrenal glomerulosa cells from STZ-induced diabetic rats show the decreased response of pregnenolone and aldosterone production to Ali, but the number and affinity of the Ali receptors were similar in the cells from control and diabetic rats, and concluded that the main defect responsible for adrenal dysfunction may be located at some step(s) mediating between Ali receptors and conversion of cholesterol to pregnenolone, with a disturbance downstream from Ali binding. Since the stimulation of aldosterone production in the adrenal zona glomerulosa by Ali is initiated via the phosphatidylinositol turnover pathway after binding to its receptors (Hunyady et al. 1982; Woodcock et al. 1988) we investigated the formation of IP₃, the cleavage products of phosphatidylinositol, to elucidate the mechanism responsible for reduced Ali response in the diabetic rats for the first time. Basal and Ali-stimulated IP₃ and IP₆ levels among control rats, untreated and insulin-treated diabetic rats were not different and the addition of insulin did not change Ali-induced IP₃ formation. These results suggest that the main defect responsible for altered Ali effects may be located at some steps mediating Ali action downstream from IP₃ formation.

The mechanism accounting for this defect, however, remains to be determined. Recently Nadler et al. (1990) reported that reduced acti-
vation of the 12-lipoxygenase pathway, one possible pathway utilized by Al in stimulating aldosterone production (Nadler et al. 1987), may be an important mechanism for the alteration of Al action in the diabetic rats. It might be argued that the lack of difference in IP formation in the present study may be attributable to changing cellular function induced by overnight incubation of diabetic cells in low-glucose media. This possibility, however, can be excluded by our observation that although after overnight incubation, aldosterone production from the cells of both diabetic and control rats were markedly decreased, the cells from diabetic rats still show less aldosterone response to Al than those from control rats (1.9 vs. 5.1 before and 1.2 vs. 4.5 folds increase above basal level after overnight incubation, n = 2). Further studies are needed to define the exact mechanisms of altered Al response in adrenal glomerulosa cells from the STZ-induced diabetic rats.

In summary, 2 weeks after an injection of STZ our diabetic rats had normal plasma renin activity and aldosterone level. The present in vitro study shows that altered Al response in diabetic rats may be secondary to chronic insulin deficiency and a short-term incubation of adrenal glomerulosa cells with insulin results in no changes in aldosterone production. Although the mechanism whereby chronic diabetes causes altered Al response is unclear from the present study normal IP generation in diabetic rats suggests the main defect accounting for attenuated Al effects may be located at some step(s) mediating Al action downstream from IP formation.

REFERENCES


Marques M, Bello AA, Machado VLA. In vitro specific uptake of labeled insulin by liver, adipose tissue, pituitary and adrenals in the Turtle 'Chrysemys durabigni'. Gen Comp Endocrinol 1982; 48:89-97

Nadler JL, Natarajan R, Stern N. Specific action


Penhoat A, Chatelain PG, Jaillard C, Saez JM. Characterization of insulin-like growth factor I and insulin receptors on cultured bovine adrenal fasciculata cells. Role of these peptides on adrenal cell function. Endocrinology 1988; 122:2518-26


Rocchini AP, Moorehead C, DeReme S, Goodfriend T, Ball DL. Hyperinsulinemia and the aldosterone and pressor responses to angiotensin II. Hypertension 1990; 15:861-866


Wilkes BM. Reduced glomerular angiotensin II receptor density in diabetes mellitus in the rat: Time course and mechanism. Endocrinology 1987; 120:1291-98

Woodcock EA, Smith AI, White LBS. Angiotensin II-stimulated phosphatidylinositol turnover in rat adrenal glomerulosa cells has a complex dependence on calcium. Endocrinology 1988; 122:1053-59