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

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= 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 [^{3}H] 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^{-8}M)-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^{-8}M), 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. AII (10^{-8}M)-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*

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INTRODUCTION

It has been recognized that diabetic rats induced by an injection of STZ develop hyporeninemic hypoaldosteronism during the progression of diabetes and insulin treatment can reverse the STZ-evoked effects(Hayashi *et al.* 1984; 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 hypoaldosteronism in diabetic rats. Several studies have revealed that the in vivo responses of aldosterone production to physiologic agonists such as AII, 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 AII but not to ACTH in adrenal glomerulosa cells from STZ-induced diabetic rats with hypoaldosteronism. Nadler *et al.* (1990) reported that whereas AII-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 AII response in diabetic rats. One study has shown that the formation of 12-hydroxyeicosatetraenoic acid which is a 12-lipooxygenase product and one possible mediator of AII-induced aldosterone synthesis, was attenuated in diabetic rats (Nadler *et al.* 1990). Other important intracellular mediators of AII action,however, were not investigated in that study. Recently Azukizawa *et al.* (1991) observed the number and affinity of AII receptors were normal in the diabetic rats with hypoaldosteronism and speculated that the main defect responsible for the hypoaldosteronism may be located at some steps downstream from AII 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; Pillion *et al.* 1988) including zona glomerulosa(Bergeron *et al.* 1980). Furthermore, during a short-term infusion of insulin AII 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 AII-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 AII in stimulating aldosterone production(Hunyady *et al.* 1982; Woodcock *et al.* 1988) and also determined the acute effect of insulin on AII-induced inositol phosphate formation.

MATERIALS AND METHODS

STZ and crude collagenase(type I) were obtained from Sigma Chemical Co. (St. Louis, MO); synthetic AII (human) from Bachem (Switzerland), synthetic ACTH-(1-24) from Organon (Nederland), insulin from GibcoBRL (Grand Island, NY) and [^{3}H]-myoinositol from New England Nuclear (Boston,MA). All other chemicals were purchased from Sigma Chemi-

cal Co. (St. Louis, MO).

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, (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 nondiabetic control and the noninsulin-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 seperated, 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 10mM glucose with the potassium concentration adjusted to 3.5 mM (0.1% BSA-KRH) . KRH buffer contained 135mM NaCl, 2.5mM KCl, 1mM KH_2PO_4, 1mM $MgSO_4$, 1.25mM $CaCl_2$, 10mM glucose and 20 mM HEPES(pH 7. 4). After incubation, the cells were dispersed mechanically, filtered through nylon mesh(pore size, 200 um) 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. Cell were counted in a hemocytometer and cell viability was assessed by trypan blue exclusion. Over 90% of cells excluded trypan blue and fasciculata cells were contaminated in less than 10% of glomerulosa cells in this cell preperation.

To determine the responses of aldosterone production to AII, K^+, ACTH and insulin, the glomerulosa cells (1x 10^5 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 AII, 8.7mM K^+, 10^{-8}M ACTH or 1mU/ml insulin. In this experiment, 10^{-8} M AII, 8.7mM K^+ and 10^{-8}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 1mU/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 aldoaterone level(or aldosterone in the media) were measured directly by radioimmunoassay using commercial kits from Sorin Biomedica(Italy) and Diagnostic Products Corperation(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 $NaHCO_3$, 0.1% BSA and 100 U/ml each of penicillin and streptomycin. [^{3}H] myoinositol (10 uCi/ml; 0.55uM) was added, and cells(1.5-2.0 x 10^6/ 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. [^{3}H] myoinositol-labeled cells (5-7x10^5) were incubated in plastic tubes in 0.3 ml KRH containing 10 mM LiCl and 10 minutes later 10^{-8} 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% $HClO_4$. 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). $KClO_4$ 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(IP_1) were eluted with 12ml of 0.2 M ammonium formate-0.1 M formic acid, inositol bisphosphate (IP_2) with 12ml of 0.4 M ammonium formate-0.1 M formic acid and inositol triphosphate(IP_3) with 12ml of 1 M ammonium formate-0.1 M formic acid. The column was calibrated,prior to the experiment, with tritiated standards of IP_3(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 1. Characteristics of the study animals

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

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.

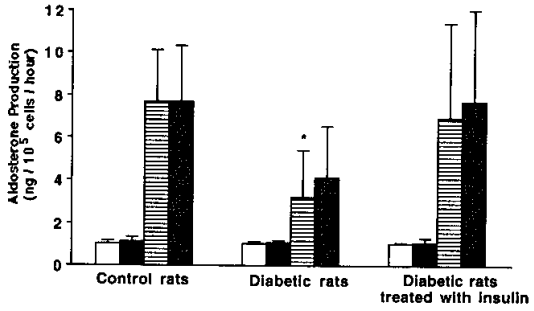

Fig. 1. Responses of aldosterone production to angiotensin II(AII, 10^{-8}M) and/or insulin (1mU/ml) in adrenal zona glomerulosa cells from control, untreated and insulin-treated diabetic rats (n=5). Bar denotes S.D., * : P<0.05 vs. control rats. □ Basal, ■ Insulin(1mU/ml), ▤ AII(10^{-8}M), ▨ AII(10^{-8}M)+Insulin(1mU/ml)

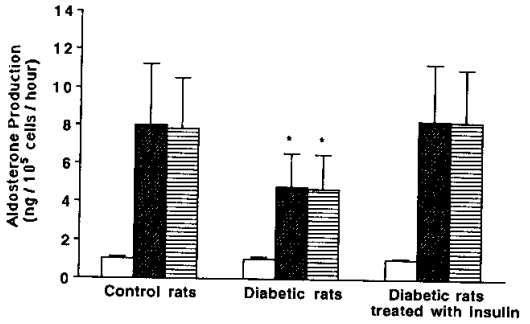

Fig. 2. Responses of aldosterone production to potassium (K^+) alone (8.7mM) and potassium (8.7mM) plus insulin (1mU/ml) in adrenal zona glomerulosa cells from control, untreated and insulin-treated diabetic rats (n=5). Bar denotes S.D., * : 0.05<P<0.1 vs. control and insulin-treated rats. □ Basal, ▨ K^+(8.7mM), ▤ K^+(8.7mM)+Insulin(1mU/ml)

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 AII alone or AII 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 AII 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/10^5 cells/ hr ,P<0.05). This effect of the diabetic state on AII-stimulated aldosterone production was not observed with insulin treatmeant (6.9±4.5 ng/10^5 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 AII 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 AII-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/10^5 cells /hr and 4.7±1.8 vs 7.8±2.7, 8.2±2.8 ng/10^5 cells /hr, respectively), as shown in Fig. 2. The difference, however, was not statistically significant (0.05<P<0.1). 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.

Incubation of [^{3}H] myoinositol- labeled

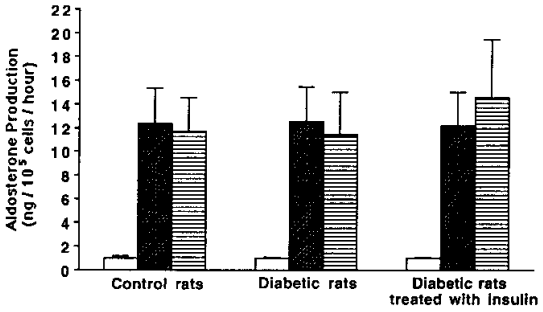

Fig. 3. Responses of aldosterone production to ACTH (10^{-8}M) alone and ACTH (10^{-8}M) plus insulin (1mU/ml) in adrenal zona glomerulosa cells from control, untreated and insulin-treated diabetic rats (n=5). Bar denotes S.D.. There were no significant differences in aldosterone production among the three groups.
□ Basal, ▨ ACTH (10^{-8}M),
▤ ACTH (10^{-8}M) + Insulin (1mU/ml)

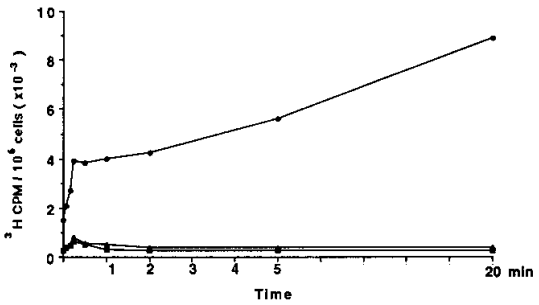

Fig. 4. Time course of accumulation of inositol phosphate in [^{3}H] myoinositol-labeled adrenal zona glomerulosa cells in the presence of 10^{-8}M angiotensin II and 10mM LiCl. The experiment was perrformed three times using 2.5-3.0x10^6 cells/each time.
● IP_1 : inositol monophosphate
△ IP_2 : inositol bisphosphate
■ IP_3 : inositol triphosphate

Table 2. Inositol phosphate response to angiotensin II and insulin

	Control rats			Diabetic rats			Diabetic rats treated with insulin		
	Basal	AII	AII+Ins	Basal	AII	AII+Ins	Basal	AII	AII+Ins
IP_1	2075 ±1113	5253 ±2870	5280 ±2740	2092 ±1086	5331 ±2765	5223 ±2822	2198 ±983	5886 ±2467	6499 ±1949
IP_2	551 ±238	1507 ±809	1510 ±738	570 ±204	1569 ±892	1575 ±844	558 ±224	1569 ±809	1559 ±746
IP_3	296 ±118	631 ±241	623 ±257	286 ±97	657 ±218	686 ±200	278 ±113	630 ±244	649 ±245

Values given are mean±S.D.. Results shown are inositol phosphate accumulation in counts per min (mean±S.D.)10^6 cells at 30 seconds. The experiments was performed five times, using 1.5~2.0×10^6 cells/ml/group. AII : 10^{-8} M angiotensin II, Ins : 1 mU/ml insulin IP_1 : inositol monophosphate, IP_2 : inositol bisphosphate, IP_3 : inositol triphosphate. There were no differences in inositol phosphate response among the three groups.

adrenal glomerulosa cells with AII (10^{-8}M) and LiCl (10mM) resulted in rapid increases in the levels of IP_1,IP_2 and IP_3. As shown in Fig. 4, maximal increases in IP_2 and IP_3 were detected as early as 15 sec whereas IP_1 increases continuously during 20 min after the addition of 10^{-8}M AII . Because IP_1,IP_2 and IP_3 remained elevated significanly above basal level 30 sec after the addition of AII, these inositol phosphates were measured at 30 sec after the addition of AII and insulin. As shown in table 2, basal IP_1,IP_2 and IP_3 levels were not different among control , untreated and insulin-treated diabetic rats and incubation with 10^{-8}M AII resulted in similar increases in these inositol phosphates levels. AII- 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 AII 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 funtion: 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 AII- 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 7-60 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 AII but not to ACTH in the present study, suggesting that the diabetic rats may have selective impairment of glomerulosa cells to AII because the mechanism of actions of AII 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 attendent AII deficiency may be responsible for selective impairment of glomerulosa cells to AII(Kigoshi *et al.* 1986; Rebuffat *et al.* 1988). Since AII 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 AII could be responsible for this selective impairment. The results of normal plasma renin activity and its resulting possibly normal plasma AII in the present study , however,do not exclude the possibility that chronic AII deficiency may cause the decreased aldosterone responses in the diabetic rats. Accumulating evidence shows that AII produced locally from adrenal tissue is important in aldosterone pro-

duction and the concentration of local AII is quite different from that of blood AII (Parkinson *et al.* 1984; Kifor *et al.* 1991). However, our results of normal plasma renin activity and altered AII response suggest that chronic lack of insulin may play a more important role than chronic deficiency of AII 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 hyporeneninemic hypoaldosteronism ,as well as altered K^+ response and speculated that such subnormal response of aldosterone production to K^+ in the diabetic rats may be due to chronic deficiency of AII since there is strong evidence that an AII-K^+ interdependency is important in their seperate 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 AII 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 AII 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 (1mU/ml) potentiated AII-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) AII infusion augments aldosterone secretion. This difference may be related to the differences of the experiment condition since we used rats and perfomed 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 AII 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 AII , but the number and affinity of the AII 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 AII receptors and conversion of cholesterol to pregnenolone, with a disturbance downstream from AII binding. Since the stimulation of aldosterone production in the adrenal zona glomerulosa by AII 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 IP ,the cleavage products of phosphatidylinositol, to elucidate the mechanism reponsible for reduced AII response in the diabetic rats for the first time. Basal and AII-stimulated IP_1,IP_2 and IP_3 levels among control rats, untreated and insulin-treated diabetic rats were not different and the addition of insulin did not change AII-induced IP formation. These results suggest that the main defect responsible for altered AII effects may be located at some steps mediating AII action downstream from IP 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-lipooxygenase pathway, one possible pathway utilized by AII in stimulating aldosterone production(Nadler *et al.* 1987), ma y be an important mechanism for the alteration of AII 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 AII 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 AII 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 AII 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 AII response is unclear from the present study normal IP generation in diabetic rats suggests the main defect accounting for attenuated AII effects may be located at some step(s) mediating AII action downstream from IP formation.

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