Effect of Administration of Insulin on Streptozotocin-Induced Diabetic Hypertension in Rat

Shanwan Chen, Christina M. Yuan, Francis J. Haddy, Motilal B. Pamnani

Abstract

We have previously shown that hypertension regulated develops when rats with 25% reduced renal mass (RRM) are treated with streptozotocin and that this hypertension is associated with albuminuria, increased extracellular fluid volume (ECFV), and the appearance in plasma of an Na+,K+-ATPase inhibitor and that the albuminuria, volume expansion, appearance of the Na+,K+-ATPase inhibitor, and hypertension can be prevented by treatment with insulin at the time the IDDM is induced, ie, if the insulin treatment is started before the development of hypertension. In the latter study, neutral protamine Hagedorn (NPH) insulin treatment (initially 4 IU/d for 2 to 3 days and then 6 IU/d) immediately after streptozotocin injection prevented the hypertension and all the other changes, even though blood glucose was not completely normalized. More, insulin treatment did not reverse the increase in plasma Na+,K+-ATPase inhibitory activity (determined by both radioimmunoassay and bioassay) and the inhibition of myocardial microsomal Na+,K+-ATPase activity observed in the untreated diabetic hypertensive rats. S'-Nucleotidase, a membrane marker, was not different between insulin-treated and untreated diabetic rats. These results show that insulin, given as here described, does not reverse the insulin-dependent diabetes mellitus hypertension in 25% reduced renal mass rats once it is established, perhaps because it does not reverse the albuminuria, volume expansion, increase in endogenous digoxin-like substance, and inhibition of cardiovascular muscle cell Na+,K+-ATPase activity. (Hypertension. 1994;23[part 2]:1046-1050.)

Key Words • insulin • digitalis • hypertension, experimental • diabetes mellitus • streptozotocin

The role of insulin in blood pressure regulation is complicated. Insulin increases renal sodium absorption and sympathetic nerve activity, both of which tend to elevate arterial pressure. On the other hand, insulin stimulates Na+ K+ pump activity, thereby causing electrogenic hyperpolarization of the vascular muscle cell and vasodilation. Clinically, insulin treatment has no significant effect on insulin-dependent diabetes mellitus (IDDM) hypertension. Chronic administration of insulin in experimental IDDM animals has been shown to increase, decrease, and have no effect on blood pressure.

We have previously shown that hypertension regularly develops when rats with 25% reduced renal mass (RRM) are treated with streptozotocin and that this hypertension is associated with albuminuria, increased extracellular fluid volume (ECFV), and the appearance in plasma of an Na+,K+-ATPase inhibitor and that the albuminuria, volume expansion, appearance of the Na+,K+-ATPase inhibitor, and hypertension can be prevented by treatment with insulin at the time the IDDM is induced, ie, if the insulin treatment is started before the development of hypertension. In the latter study, neutral protamine Hagedorn (NPH) insulin treatment (initially 4 IU/d for 2 to 3 days and then 6 IU/d) immediately after streptozotocin injection prevented the hypertension and all the other changes, even though blood glucose was not completely normalized. More, insulin treatment did not reverse the increase in plasma Na+,K+-ATPase inhibitory activity (determined by both radioimmunoassay and bioassay) and the inhibition of myocardial microsomal Na+,K+-ATPase activity observed in the untreated diabetic hypertensive rats. S'-Nucleotidase, a membrane marker, was not different between insulin-treated and untreated diabetic rats. These results show that insulin, given as here described, does not reverse the insulin-dependent diabetes mellitus hypertension in 25% reduced renal mass rats once it is established, perhaps because it does not reverse the albuminuria, volume expansion, increase in endogenous digoxin-like substance, and inhibition of cardiovascular muscle cell Na+,K+-ATPase activity. (Hypertension. 1994;23[part 2]:1046-1050.)

There were three groups: (1) 25% RRM alone, (2) 25% RRM plus streptozotocin diabetes, and (3) 25% RRM plus streptozotocin plus insulin. Blood pressure rose in the untreated group 2 but not in the insulin-treated group 3 (blood pressure was not different from that in the control group 1 animals). Body weight increased at a normal rate in the treated group 3 but not in the untreated group 2. Albuminuria, which occurred in the untreated group, did not occur with insulin treatment. Left ventricular microsomal Na+,K+-ATPase activity, which was suppressed in the untreated group, was normal in the insulin-treated group. The same was the case for Na+,K+-ATPase activity of right ventricular microsomes. The Na+,K+-ATPase inhibitory activity in the plasma remained normal with insulin treatment, as did plasma digoxin-like immunoreactive factor, plasma renin activity (PRA), ECFV, left ventricular weight, and kidney weight, even though blood glucose was not completely normalized (203 mg/dL).

In the present study we examined the effect of essentially the same insulin treatment on established hypertension in streptozotocin-induced IDDM rats with 25% RRM; ie, the hypertension was allowed to develop before insulin was given. This posttreatment failed to ameliorate the hypertension and most of the changes associated with it.

Methods

Experimental Animals

Male Wistar rats weighing 250 to 280 g (Charles River) with documented normotension underwent 25% surgical RRM. Briefly, the lower poles of both kidneys (each 12.5% of total renal mass) were removed through a midline abdominal
incision. This was achieved by encircling the lower pole of each
kidney with a loop of No. 0000 silk suture and then tightening
the loop. This method both cut the tissue and tied off the
vessels. After 2 weeks of surgical recovery and documented
normotension, when body weight was 350 to 390 g, the animals
were entered into the study.

IDDM was induced by the jugular vein injection of 65 mg/kg
body wt streptozotocin (Sigma Chemical Co) in citrate buffer
saline, pH 4.5. After 4 weeks of sustained diabetes and
hypertension, NPH insulin (Eli Lilly & Co) (6 to 8 IU) was
subcutaneously administered each day. The final injection
was given the day before the animals were killed. Three groups
of rats were studied: (1) 25% RRM rats with streptozotocin-
induced diabetic hypertension treated with insulin (25-DM-
In), (2) 25% RRM rats with streptozotocin-induced IDDM
and hypertension (25-DM), and (3) 25% RRM rats receiving
vehicle (citrate saline) (25-V). All rats were given tap water
and a standard rat chow diet (Biosevis Inc) ad libitum.

Blood pressure was monitored weekly by tail plethysmography
(Natsume KN 209). Eight weeks after streptozotocin (4 weeks
after insulin treatment) or vehicle treatment, blood pressure
was recorded directly with rats under pentobarbital anesthesia,
blood samples were collected, and the hearts and kidneys were
removed for membrane preparation.

Membrane Preparation
Microsomal fractions were prepared from ventricles and
renal medullas as described previously. Briefly, the right and
left ventricles and renal outer medulla were each minced with
a scalpel and homogenized with a motor-driven polytetrafluoro-
ethylene pestle in 10 mL deoxycholate solution at pH 6.8.
The homogenate was centrifuged (SM-24 rotor) at 1100g for
10 minutes to remove the nuclear fraction and at 12,300g for
20 minutes to remove the mitochondrial fraction. The superna-
tant was then centrifuged at 35 000g for 1.5 hours to obtain the
microsomal fraction. The microsomal fraction was treated with
1 mol/L NaI solution and recovered in 10 mmol/L imidazole-
HCl (pH 7.4) solution.

Enzyme Activity Assays
Membrane ATPase activity was assayed by measuring the
amount of inorganic phosphate liberated from ATP (Tris-
ATP, Sigma) during a 1-hour incubation at 37°C. The assay
medium for total ATPase activity contained 2 mL (mmol/L)
NaCl 120, KCl 10, Tris-HCl 40 (pH 7.5 at 37°C), Tris-ATP 2,
MgCl2 2.5, and EGTA 0.5. Mg2+ATPase activity was
assayed under the same conditions except that KCl was omitted
and replaced by 1 mmol/L ouabain. Na+, K+-ATPase activity
was calculated by subtracting Mg2+-ATPase activity from total
ATPase activity. 5'-Nucleotidase activity was assayed by the method
of Emmelot et al.a

Radioimmunoassay for Plasma Digoxin-Like
Immunoreactive Factor
Plasma from both experimental and control rats was ex-
tracted as described previously. One millilitre of plasma and
2 mL ethanol were mixed and boiled at 95°C for 5 minutes.
The supernatant was centrifuged at 35 000g for 20 minutes,
dried and dissolved in 1 mL deionized water, and then eluted
using C18 cartridges (Millipore). The C18 columns were
prewashed with 5 mL methanol and then with 10 mL water.
After extraction of the deproteinized plasma samples, the
columns were washed with 10 mL water and then eluted with
4 mL methanol. The methanol fractions were dried and
reconstituted with deionized water. The digoxin-like immuno-
reactive factor was estimated using a commercially available
digoxin radioimmunoassay (IncStar).a

Plasma Bioassay for Digitalis-Like Substance
Plasma from experimental and control rats was bioassayed
for digitalis-like substance using a canine kidney Na+, K+-
ATPase preparation as described previously. Briefly, 1 mL
plasma and 2 mL ethanol were mixed and boiled at 95°C for
5 minutes. The supernatant was dried and dissolved in 1 mL
deionized water and then applied to C18 cartridges. The
columns were washed with 8 mL water and then eluted with
2.5 mL of 50% ethanol. The ethanol fraction was dried and
reconstituted with deionized water. Canine microsomal
ATPase was freshly prepared from normal dog kidney as
described previously. The final medium for ATPase activities
was the same as described above, except that 0.2 mmol/L
ouabain was added for Mg2+-ATPase activity. Percent inhibition
was calculated as (1—Ap/AQ)×100%, where AQ and AP are
Na+, K+-ATPase activity with or without plasma, respectively.

Other Measurements
Just before animals were killed, blood pressure and heart
rate were recorded with animals under pentobarbital (50
mg/kg) anesthesia through a PE-50 carotid artery catheter
using a pressure transducer (model P23Db, Gould Statham).
In some animals, ECFV and PRA were determined 8 weeks
after streptozotocin (4 weeks after insulin treatment) or
vehicle injection. ECFV was measured using sodium thio-
cyanate as described previously. PRA was measured using
a commercially available kit (IncStar). Urinary albumin excre-
tion was determined in a 24-hour urine collection using an
enzyme-linked immunoassay. Plasma glucose was measured
by a glucose analyzer (Beckman Instruments).

Data Analysis
All data are expressed as mean±SEM. Statistical signifi-
cance was determined using a two tailed t test for comparing
the means of independent samples. ANOVA was used to
detect possible significant differences among groups. For re-
peated measurements in a given group, ANOVA was used
followed by Duncan’s multiple range test to determine the
significance of the F ratio. A value of p<.05 was considered
significant.

Results
After intravenous injection of streptozotocin, all 25%
RRM rats developed diabetes (as indicated by hyper-
glycemia [see the Table]) and an increase in blood
pressure (Fig 1A). This was temporally correlated with
albuminuria (Fig 1B). The 25-V rats remained normo-
motensive and normoalbuminuric throughout (Fig 1A
and 1B). After insulin treatment the 25-DM-In rats had
lower blood glucose levels and plasma osmolality than
25-DM rats (Table). The 25-DM-In rats also gained
weight, whereas the 25-DM rats did not during the
insulin treatment (Fig 1C). However, there were no

Results
After intravenous injection of streptozotocin, all 25% RRM rats developed diabetes (as indicated by hyperglycemia [see the Table]) and an increase in blood pressure (Fig 1A). This was temporally correlated with albuminuria (Fig 1B). The 25-V rats remained normotensive and normoalbuminuric throughout (Fig 1A and 1B). After insulin treatment the 25-DM-In rats had lower blood glucose levels and plasma osmolality than 25-DM rats (Table). The 25-DM-In rats also gained weight, whereas the 25-DM rats did not during the insulin treatment (Fig 1C). However, there were no differences in blood pressure and urinary albumin excretion between insulin-treated and untreated diabetic rats (Fig 1A and 1B). Heart rate was significantly increased in 25-DM-In rats relative to 25-DM rats (Table). Left ventricular weight increased in both groups of diabetic rats (25-DM) relative to 25-V rats (Table). No differences were found in right ventricular weight among the three groups (Table). Kidney weight decreased in 25-DM-In rats relative to 25-DM rats, but both diabetic groups showed significant renal hypertrophy relative to 25-V rats (Table). The 25-DM-In rats had lower PRA and higher ECFV values than 25-V rats, but the values were not different from those in 25-DM rats.

The myocardial microsomal Na+, K+-ATPase activity of the left and right ventricles was significantly decreased in both 25-DM-In and 25-DM rats relative to 25-V rats. The values in 25-DM-In and 25-DM rats rats

Effects of Insulin on Diabetic Hypertension
Extracellular Fluid Volume, Plasma Renin Activity, Mean Blood Pressure, Heart Rate, and Organ Weights in 25% Reduced Renal Mass Rats Treated with Streptozotocin and Insulin

<table>
<thead>
<tr>
<th>Experimental Rats</th>
<th>Body Weight, g</th>
<th>Plasma Glucose, mg/dL</th>
<th>Plasma Osmolality, Osm/kg</th>
<th>ECFV, mL/100 g body wt</th>
<th>PRA, (ng Ang l/mL)/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-DM-In (n=10)</td>
<td>455±13*</td>
<td>218±8.8†</td>
<td>309±2.8*</td>
<td>21±0.49†</td>
<td>9.89±2.2†</td>
</tr>
<tr>
<td>25-DM (n=9)</td>
<td>357±12†</td>
<td>506±10.5‡</td>
<td>328±2.4†</td>
<td>23.1±1.3‡</td>
<td>7.41±1.4‡</td>
</tr>
<tr>
<td>25-V (n=9)</td>
<td>476±12</td>
<td>153±6.3</td>
<td>301±1.4</td>
<td>16.8±0.27</td>
<td>20.1±3.1</td>
</tr>
</tbody>
</table>

ECFV indicates extracellular fluid volume; PRA, plasma renin activity; Ang I, angiotensin I; MBP, mean blood pressure; LV, left ventricle; RV, right ventricle; 25-DM-In, 25% reduced renal mass rats treated with streptozotocin and insulin; 25-DM, with streptozotocin alone; and 25-V, with vehicle. Values are mean±SEM.

*P<.05 25-DM-In vs 25-DM rats.
†P<.05 25-DM-In vs 25-V rats.
‡P<.05 25-DM vs 25-V rats.

were not significantly different (Fig 2A and 2B). The myocardial Mg\(^{2+}\)-ATPase and S\(^{-}\)-nucleotide activities were not different in the three groups of animals (Fig 2A and 2B). Renal medullary Na\(^{+}\), K\(^{+}\)-ATPase activity of both diabetic groups was significantly greater than that of 25-V rats (Fig 2C). However, the renal Na\(^{+}\), K\(^{+}\)-ATPase activity of 25-DM-In rats was significantly lower than that in 25-DM rats (Fig 2C). Mg\(^{2+}\)-ATPase and S\(^{-}\)-nucleotide activities of kidney were not different among the three rat groups. Plasma digoxin-like immunoreactive factor (measured by radioimmunoassay) and digitalis-like substance were elevated in both diabetic rat groups relative to 25-V rats, but they did not differ in the two groups of diabetic animals (Fig 3A and 3B).

**Discussion**

These studies show that in this model of diabetic hypertension insulin in the amount given does not affect blood pressure once the hypertension is established. This contrasts with our previous study\(^7\) in which early treatment with essentially the same amount of insulin prevented the development of hypertension. How do we explain the difference? In our previous study early treatment prevented the appearance of albuminuria, increased ECFV, depressed PRA, increased plasma Na\(^{+}\), K\(^{+}\)-ATPase inhibitor, and depressed myocardial Na\(^{+}\), K\(^{+}\)-ATPase activity. In the current study insulin had no effect on these parameters; ie, once the albuminuria, increased ECFV, decreased PRA, increased plasma Na\(^{+}\), K\(^{+}\)-ATPase inhibitor, and decreased myocardial Na\(^{+}\), K\(^{+}\)-ATPase activity had appeared, they were not affected by the insulin treatment. This was the case despite the fact that insulin treatment partly corrected plasma and urine glucose levels and increased body weight.

One possibility is that nephropathy, once established, is not reversed by insulin. Albuminuria is thought to be an index of diabetic nephropathy.\(^9\) Certainly the albuminuria did not improve with insulin treatment. The ability to excrete sodium and water might not have increased either. This would account for the sustained increase in ECFV, which is thought to be the stimulus for release of digitalis-like substance.\(^10,11\) The insulin treatment did partly reverse the renal hypertrophy and the increased renal Na\(^{+}\), K\(^{+}\)-ATPase activity (thought to result from increased single-nephron glomerular filtration rate and sodium load) seen in this model.\(^5,7\) The latter would be expected to decrease tubular sodium...
Continued

<table>
<thead>
<tr>
<th>MBP, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>LV, g/100 g body wt</th>
<th>RV, g/100 g body wt</th>
<th>Kidney, g/100 g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>137±5.7†</td>
<td>330±13†</td>
<td>0.220±0.002†</td>
<td>0.05±0.001</td>
<td>0.68±0.08†</td>
</tr>
<tr>
<td>132±4.8†</td>
<td>284±10†</td>
<td>0.228±0.004†</td>
<td>0.05±0.004</td>
<td>0.91±0.16†</td>
</tr>
<tr>
<td>109±3.6</td>
<td>383±12</td>
<td>0.186±0.001</td>
<td>0.05±0.001</td>
<td>0.54±0.05</td>
</tr>
</tbody>
</table>

Reabsorption and was thought to have played a role in preventing the development of hypertension in our earlier study. However, the same changes in the present study did not ameliorate the hypertension. The low PRA in the insulin-treated animals most likely is the result of sodium and water retention because insulin per se stimulates renin secretion. 17 In humans, insulin treatment has been shown to have no effect on hypertension associated with IDDM. 1 We acknowledge that in our study we achieved only moderate glycemic control, which might not have been sufficient to reverse the observed changes, if that is indeed possible.

Increased plasma digitalis-like substance was demonstrated by both radioimmunoassay and bioassay. Myocardial Na⁺,K⁺-ATPase activity decreased, and the extent of the decrease was not influenced by insulin (even though insulin has the propensity to increase Na⁺,K⁺-ATPase activity). The decrease in myocardial Na⁺,K⁺-ATPase activity cannot be secondary to changed microsomal distribution caused by ventricular hypertrophy; the left and right ventricles had the same changes in Na⁺,K⁺-ATPase activity even though the right ventricle was not hypertrophied. Furthermore,
5'-nucleotidase, a plasma membrane marker, was not different among the three groups. Inhibition of Na\(^+\),K\(^+\)-ATPase appears to increase blood vessel and heart contractility and raise blood pressure.\(^{13,15}\)

Insulin partly corrected the bradycardia seen in the 25-DM rats. This might reflect increased sympathetic nervous system action. Insulin also may have increased the sensitivity of the heart to catecholamines.\(^{16}\)

In summary, even though early insulin treatment prevents the development of hypertension in our model of diabetes hypertension, it fails to ameliorate the hypertension once established. Only one insulin regimen was studied; others should also be explored.

References


Effect of administration of insulin on streptozotocin-induced diabetic hypertension in rat.
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