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# Insulin stimulates renal glomerular sodium-potassium adenosine triphosphatase activity

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**The effect of insulin on total and ouabain-inhibited membrane-bound adenosine triphosphatase (ATPase) activity in renal glomeruli isolated from adult white rats was examined. In concentrations of 1–10 μg/ml, insulin significantly stimulated the ouabain-inhibited $(Na^+ + K^+)$-ATPase activity, without affecting total (composite) ATPase activity. These results, coupled with previous findings demonstrating that glomerular $(Na^+ + K^+)$-ATPase activity is reduced in acute streptozotocin diabetes, suggest that the renal glomerulus is a target tissue with respect to this biologic effect of insulin.**

The ubiquitous sodium pump of mammalian cells, which serves to promote the active influx of potassium and extrusion of sodium, is dependent on the enzyme sodium-potassium adenosine triphosphatase ($(Na^+ + K^+)$-ATPase) [1–4]. The activity of this enzyme appears to be modulated by various hormones in a variety of tissues in different animals. For example, thyroid hormone stimulates $(Na^+ + K^+)$-ATPase activity in rat liver, kidney, and skeletal muscles [5–8], while insulin stimulates the enzyme in frog sartorious muscle [9], rat hepatoma cells [10] and hepatocytes [11], and also promotes $(Na^+ + K^+)$-ATPase-dependent rubidium entry into rat adipocytes [12]. We became interested in the effect of insulin on $(Na^+ + K^+)$-ATPase activity in the kidney, and in specific renal sites, in view of our recent demonstration that the $(Na^+ + K^+)$-ATPase activity associated with membranes prepared from glomeruli isolated from rats with acute streptozotocin diabetes is decreased compared to activity in preparations from control animals [13,14]. Although the significance of this reduced glomerular $(Na^+ + K^+)$-ATPase activity with respect to changes in renal sodium handling or the glomerular lesions accompanying diabetes is not yet clear, it may represent an absence of insulin effect on a target tissue. These considerations suggested that insulin might directly stimulate glomerular $(Na^+ + K^+)$-ATPase activity. Furthermore, insulin receptors are present in glomerular cells [15], and there is some evidence from studies in other tissues that the hormone's influence on $Na^+/K^+$ pump activity occurs proximal to or mediates the interaction of insulin with its receptor [10,16]. We therefore examined the effect of insulin in vitro on $(Na^+ + K^+)$-ATPase activity in isolated rat renal glomeruli.

Glomeruli were isolated from adult male white rats by sieving through a series of stainless steel meshes yielding preparations virtually free of tubular elements [17]. Crude membrane fractions were prepared by hypotonic lysis in 10 mM Tris-HCl followed by repeated washing and collection by centrifugation [18]. After overnight storage at −70°C, $(Na^+ + K^+)$-ATPase activity was measured in thawed samples as the ouabain-inhibited inorganic phosphorus released on incubation of the membranes with ATP as previously described

[14]. The incubations were conducted in 1–2 ml of assay solution containing the indicated concentrations of the following: 100 mM NaCl, 10 mM KCl, 1 mM EGTA, 5 mM $MgCl_2$, and 100 mM imidazole (pH 7.4). To inhibit $(Na^+ + K^+)$-ATPase activity, KCl was omitted and 1.0 mM ouabain added. Phosphorus was measured by the method of Fiske and SubbaRow [19]. Purified bovine insulin (Sigma Chemical Co.), when present, was added at the start of the incubation period to achieve concentrations of 0.1–10 μg/ml. Protein was measured by the method of Bradford [20].

Preliminary experiments established that glomerular membrane ATPase activity, both total and ouabain-inhibited, showed a direct relationship with time between 0 and 10 minutes of incubation, and with protein concentration up to 20 μg per assay [14]. Between 20–30% of total glomerular ATPase activity was inhibited by 1 mM ouabain; further inhibition was not observed with higher concentrations of ouabain. These conditions were therefore used for the present experiments. Each series of incubations without and with ouabain, and in the absence or presence of insulin, was performed with lysed glomerular preparations obtained from the same animal. All incubations were conducted in duplicate, and each used two concentrations of protein (5 and 10 μg) and two time periods (5 and 10 min) to yield a single data point. Results were calculated as μmol phosphorus released per mg protein per min.

Table I shows the effect of insulin on the ouabain-inhibited ATPase activity of renal glomerular cell membranes. Insulin significantly stimulated activity when present during the incubation periods at concentrations of 1 and 10 μg/ml. The difference in activities observed between control and 100 ng/ml of insulin was not significant, but the differences between control versus 1 μg/ml and between 1 versus 10 μg/ml were statistically significant and the mean values at each concentration of insulin suggested a dose response effect. In contrast, composite (total) glomerular ATPase activity in the presence of insulin did not differ from control values at all concentrations tested (Table I). Thus, percent of total glomerular ATPase activity that was inhibited by ouabain rose from 23% in the absence of insulin to 35% in the presence of 10 μg/ml of insulin.

TABLE I

EFFECT OF INSULIN ON GLOMERULAR ATPase ACTIVITY

Results represent means ± S.E. of the number of experiments given in parentheses. Each experiment was conducted for two time periods with two concentrations of protein to yield a single data point, as detailed in the text. Results are expressed as umol phosphorus liberated per mg of protein per min. Composite = total activity in the presence of 10 mM KCl; $(Na^+ + K^+)$ = ouabain-inhibited activity, measured in the absence of KCl and the presence of 1.0 mM ouabain.

| Insulin | ATPase activity | |
|---|---|---|
| | Composite | $(Na^+ + K^+)$ |
| None | 2.757 ± 0.123 (10) | 0.630 ± 0.083 (10) |
| 100 ng/ml | 2.962 ± 0.300 (7) | 0.658 ± 0.075 (7) |
| 1 μg/ml | 2.780 ± 0.200 (10) | 0.762 ± 0.126 (10) * |
| 10 μg/ml | 2.833 ± 0.315 (10) | 0.986 ± 0.222 (10) * |

* $P < 0.05$ compared to control.

It is worth nothing that the absolute values for $(Na^+ + K^+)$-ATPase activity observed in control incubations in these experiments are higher than those reported for whole kidney or renal cortex by Finegold et al. (0.07 μmol/mg per min [21] and by Lo and co-workers (0.125 μmol/mg per min [22,23]), a finding that may relate to the use of isolated glomerular preparations rather than subcellular fractions of whole tissue homogenates. On the other hand, the values are comparable to those reported by Ku and Meezan (0.90 μmol/mg per min [24]), who used whole kidney homogenized in a solution containing 0.1% sodium deoxycholate. However, in a series of experiments ($n = 4$) using glomeruli homogenized in 0.1% sodium deoxycholate exactly following the method described by Ku and Meezan as enzyme source, we were unable to detect an effect of insulin on $(Na^+ + K^+)$-ATPase activity. The reason for this is not clear, but may relate to unmasking of latent activity by detergent treatment. Although freezing and thawing would be expected to fracture glomerular membranes, it is possible that the observed insulin effect on $(Na^+ + K^+)$-ATPase activity in these preparations in part derived from unmasking of residual latent sites, whereas no such sites remained in detergent-treated samples.

The insulin-induced stimulation of $(Na^+ +$

$K^+$)-ATPase activity in lysed preparations of isolated glomeruli that was observed in these experiments occurred with insulin concentrations within the range of that reported to stimulate pump activity in other tissues [10,25]. This effect is rapid and occurs within minutes after incubation of glomerular membranes with the hormone. These results strengthen the interpretation of previous experiments which showed that glomerular ($Na^+ + K^+$)-ATPase activity is significantly diminished in acute streptozotocin diabetes [13,14]. That insulin rapidly promotes stimulation of the enzyme activity in glomerular membranes is consistent with the hypothesis that the reduction in activity found in glomeruli isolated from streptozotcin-diabetic rats is a result of insulin deficiency in vivo. These findings may help explain the sodium loss seen in uncontrolled diabetes and its correction by insulin [26,27], and suggest that the renal glomerulus is a target tissue responsive to biologic effects of insulin.

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