NOTE
Increase of Urinary Insulin Excretion Following Probenecid Administration in Man

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Synopsis

Oral administration of probenecid augmented urinary clearance of, trichloroacetic acid precipitable $^{131}$I-insulin in six men who were injected with tracer dose of $^{131}$I-insulin intravenously. The increments were 16 to 423% above the control value ($P < 0.05$). In other eight men, endogenous insulin in urine and plasma was measured by radioimmunoassay method. Apparently increased responses in urinary insulin excretion and urinary insulin clearance were observed after probenecid administration. The percentage increases of the insulin excretion and the insulin clearance beyond the control values were 2 to 200 ($P < 0.01$) and 7 to 200 ($P < 0.01$), respectively.

It was suggested that insulin was reabsorbed at the proximal convoluted tubules and that probenecid affected this reabsorption mechanism.

Many investigators presented the evidence that the kidney plays a significant role in the removal of insulin from plasma. After injection of $^{131}$I-insulin into a peripheral vein, a greater proportion of radioactivity was shown to be localized in the kidney than other in any organ of rats (Elgee et al., 1954). Radioautographic studies demonstrated that the radioactivity was highly concentrated in the proximal convoluted tubules (Elgee et al., 1954; Narahara et al., 1958; Darmady, 1965). Chamberlain and Stimmmer (1967) suggested that insulin is filtered at the glomerulus and almost completely reabsorbed and degraded by cells lining the proximal tubules in man. This degrading mechanism of renal tubules on insulin may account for the findings that only a small amount of immunoreactive insulin is excreted in the urine (Jorgensen, 1966; Chamberlain and Stimmmer, 1967; Rubenstein et al., 1967). Because probenecid affects tubular transport mechanism (Weiner and Mudge, 1964), we decided to determine its effects on the excretion of $^{131}$I-labeled insulin and endogenous immunoreactive insulin by the human kidney.

Experiments

Fourteen male patients with normal renal function were studied throughout experiments I and II. None of them had received insulin injection in their histories.

Experiment 1

A dose of 20 to 80 $\mu$Ci of $^{131}$I-labeled bovine insulin which was purchased from Abott Laboratories (Specific activity; 5.87–6.38 mCi/mg) was given to six patients who had received 100 mg of potassium iodide 20 hr earlier to inhibit thyroidal iodine uptake. Blood samples were collected 15, 30, 45 and 60 min after the injection. Urine samples were collected for 2 hr after the $^{131}$I-insulin injection. After three days the study was repeated in the same patients while they were receiving 1.0 g of probenecid by mouth one hour before the $^{131}$I-insulin injection. Plasma and urine ali-
quotions were added to trichloroacetic acid (TCA) solution so that the final concentration of TCA was 15%. One ml of 5% bovine serum albumin was added to each 10 ml of urine samples as a carrier. After two washes with 20% TCA solution, the precipitated proteins were counted for radioactivity in a well type scintillation counter. Urinary $^{131}$I-insulin clearance was calculated by dividing the rate of urinary excretion by the plasma concentration of the 30-minute specimen.

Administration of probenecid augmented urinary clearance of TCA precipitable radioactivity (Fig. 1). The increments were 16 to 423% above the control values (P<0.05). No significant difference in the time courses of disappearance of $^{131}$I-insulin in plasma was obtained between the control and the probenecid experiments. Mean half time of $^{131}$I-insulin in plasma was 40 min in both experiments.

**Experiment 2**

Other eight patients ingested 100 g of glucose with and without 1.0 g probenecid that was administered one hour before the glucose ingestion. The interval of the control and the probenecid experiments was one week. Blood samples were taken 0, 30, 90 and 120 min after glucose load, and two-hour-urine specimens were also collected. Plasma glucose was measured on the Auto Analyzer by a modification of the method of Hoffman (1937). Immunoreactive insulin was measured in plasma and urine by the double antibody technique of Morgan and Lazarow (1963). Determinations on urine dilution both from the control and the probenecid experiments showed direct proportionality between the degree of dilution and the detected insulin concentrations showing that the urinary content of other substances (including probenecid) does not influence the immunological reactions. Urinary insulin clearance was calculated using the rate of urinary excretion and the mean insulin concentration for 2 hr.

Apparantly increased responses in urinary insulin excretion and urinary insulin clearance were observed after probenecid administration (Fig. 2). The percentage increases of the insulin excretion and the insulin clearance beyond the control values were 2 to 200 (p<0.01) and 7 to 200 (p<0.01), respectively. The responses of both plasma glucose and plasma insulin
after glucose load in the probenecid experiment resulted in curves almost identical to those for the control experiment in each case.

**Discussion**

It is shown in our experiment that probenecid enhanced the urinary insulin excretion and the urinary insulin clearance without any marked change in insulin secretion or in insulin half time in plasma.

The measure of TCA precipitable activity as a measure of intact insulin in serum or urine may be a little questionable. But the almost identical results obtained on clearance of endogenous insulin may indicate the isotope technique to be satisfactory.

Probenecid is known to decrease the renal tubular secretion of several organic substances or to decrease the renal tubular reabsorption of some other organic substances through its inhibiting mechanism on p-aminohippurate (PAH) transport system (Weiner and Mudge, 1964). So, our findings indicate that probenecid inhibits the renal tubular reabsorption of insulin, and that the urinary insulin is excreted mainly by the passage through glomerules not by the renal tubular secretion. These results agree with the suggestion of others (Chamberlain and Stimmmer, 1967) that the renal tubular secretion of insulin does not occur significantly. It is reported that the urinary insulin clearance equaled or approached glomerular filtration rate (GFR) in patients with severe tubular disease (Chamberlain and Stimmmer, 1967).

Since in our experiment the maximum value of the urinary insulin clearance after probenecid load was only 0.84 ml per minute that is far below GFR, the inhibitory effect of probenecid upon tubular insulin reabsorption is thought to be of limited degree.

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**References**


