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Uric Acid-Induced Decrease in Rat Insulin Secretion (41033)[1,2]

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Abstract. Interest in the relationship of uric acid (UA) to insulin stems from reports of a possible association between gout and development of glucose intolerance as well as the structural similarity between uric acid and the diabetogen, alloxan. Previous attempts to examine this relationship using experimental animals possessing a potent uricase have produced inconsistent results. In order to examine the effects of elevated UA concentrations on serum-immunoreactive insulin (IRI), lactic acid, and glucose, we used rats treated with potassium oxonate (KOx), a competitive inhibitor of uricase, to prevent rapid degradation of UA. Male Wistar rats fed 3.5% KOx or 3.5% KOx plus 2% UA in a modified AIN-76 diet for 1 week had serum UA and whole blood lactic acid increased twofold. Hyperuricemic rats drank more water and weighed less than controls. In the 1-week experiments we describe here, animals treated with KOx alone (endogenous UA) did not become hypoinsulinemic compared to controls whereas those fed KOx *and* UA (endogenous plus exogenous UA) showed a decrease in serum IRI of 44%. In longer-term experiments (4 weeks), it was sufficient to feed 4.5% KOx alone to observe a 26% decrease in insulin. Thus, it appeared that the effect was dose and time dependent. Serum glucose increased (24–38%) in animals fed KOx/UA for 1 week. As a result, insulin:glucose ratios also decreased in the KOx/UA-fed group suggesting abnormal insulin secretion. Results from *in vitro* studies using isolated neonatal rat pancreatic islets indicated that UA, unlike KOx, rapidly suppressed insulin secretion by up to 65%. The rapidity of the suppression and subsequent reversal of the effect by removing UA from the medium further suggested that UA interferes with insulin secretion. These data indicate a reexamination is warranted of the possible cytostatic if not cytotoxic effects of UA toward the β-cells of the pancreas.

An association between gout and diabetes mellitus has been suspected for over 200 years but evidence to support such a relationship remains inconclusive (1–3). Following the discovery that the pyrimidine derivative, alloxan, could produce diabetes in experimental animals, Griffiths suggested that a purine such as uric acid (UA) with a similar chemical configuration to alloxan might be diabetogenic (4). In support of his hypothesis, Griffiths reported that administration of UA to experimental animals increased blood glucose. However, the incidence of diabetes was low in these animals and other workers were unable to confirm the UA effect (3, 5). Since most mammals except man and some of the higher apes possess the enzyme uricase which converts sparingly soluble UA to more soluble allantoin, it is remarkable that Griffiths observed any effect due to UA and not unexpected that others would have difficulty reproducing the work. In order to circumvent this problem we have treated rats with potassium oxonate (2,4-dihydroxy-6-carboxy-1,3,5-triazine; 5-azaorotic acid, KOx), a competitive inhibitor of uricase (6). With the uricase-inhibited rat as a model, we examined the effects of increased serum UA concentrations on serum-immunoreactive insulin (IRI) and selected metabolites. We also investigated the effects of physiological levels

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of UA on insulin secretion by isolated cell cultures of neonatal rat endocrine pancreas.

Materials and Methods. *Whole animal studies.* Male Wistar rats weighing 110–140 g from Canadian Breeding Farms Ltd., St. Constant, Québec were housed individually in stainless-steel cages randomly designated as (a) control (no treatment), (b) 3.5% KOx, or (c) 3.5% KOx plus 2% uric acid. Animals were maintained in temperature- and humidity-controlled rooms (22°, 50% R.H.) with lights on from 0700 to 1900 hr, fed *ad libitum* and allowed free access to water. The basic diet was a modification of the AIN-76 diet (7) consisting of the following ingredients on a percentage basis: corn starch (55.0), casein (20.0), DL-methionine (0.3), corn oil (15.0), AIN-76 mineral mix (3.5), AIN-76 vitamin mix (1.0), choline bitartrate (0.2), and a cellulose-type fiber (Alphacel, 5.0). KOx (Calbiochem, San Diego, Calif.) and uric acid (Fisher Scientific, Fairlawn, N.J.) were included in the diet at the expense of corn starch. On the sixth day, animals were fasted 16 hr overnight and then bled from the aorta while under light ether anesthesia. Serum IRI was determined using a modification of the double-antibody technique of Hales and Randle (8) with a kit supplied by Amersham Corporation (Oakville, Ontario, Canada). Serum uric acid and glucose were determined using an SMA-12/60 microauto-analyzer (Technicon Instrument Corp., Tarrytown, N.Y.). Lactic acid in whole blood was determined as described by Gutmann and Wahlefeld (9). Data were analyzed using analysis of variance and multiple comparison procedures to identify significant treatment differences (10).

Rat pancreas cultures. Pancreata were minced and enzymatically disrupted using collagenase (Type IV, Worthington Biochemical Corp., Freehold, N.J.) in Hanks' balanced salt solution (11–13). The islets of Langerhans were purified on a discontinuous density gradient of Hypaque (Winthrop, Aurora, Ontario) and Ficoll 500 (Pharmacia Canada Ltd., Montréal, Québec) at a density of 1.090 g/ml (14) with Hanks' solution layered above. After centrifugation, the islets were suspended in Medium 199 without bicarbonate but containing Hanks' salts, glutamine, 10% fetal calf serum (Grand Island Biological Co. (Gibco Canada), Burlington, Ontario), 16.7 m*M* D-glucose, 100 IU/ml sodium penicillin, 100 μg/ml streptomycin with 20 m*M* *N'*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (Hepes, Gibco Canada) present as a buffer. Plastic culture flasks were each inoculated with five to eight pancreata. After 7 days the cultures were transferred to an incubator where they were continually superfused with serum-free culture medium (5.6 m*M* D-glucose) and samples were collected at 10-min intervals. After superfusion for 12 hr, uric acid was added for 80 min. Sixty minutes later the cultures were challenged with 11.1 m*M* D-glucose. IRI was determined using a modified dextran-coated charcoal method (15, 16).

Results. Results from a typical experiment are shown in Table I. KOx and KOx/UA-treated animals consumed less food, drank more water, and weighed less at sacrifice than control animals. KOx/UA-fed animals weighed less and consumed less food than animals treated with KOx alone. In both KOx-fed groups, the internal organs weighed less on an absolute basis than those of control animals with the exception of the kidneys which remained the same in KOx-treated animals or increased in weight in animals fed KOx/UA. Kidney damage is not uncommon in animals made hyperuricemic with KOx (6). Adding KOx to the control diet increased serum UA concentration twofold. When UA was included in the KOx diet, serum UA values were not significantly higher than those from rats fed KOx alone. However, an enhancing effect of dietary UA on KOx-induced hyperuricemia is well documented (17, 18). Since KOx has a short *in vivo* half-life (2–3 hr), the 16-hr fast used in our experiments probably diminished levels of the inhibitor, resulting in decreased UA in samples taken at the time of sacrifice.

Both groups of hyperuricemic animals had serum lactic acid levels twice those of control animals (Table I). Although lactic acid is not known to suppress secretion of

TABLE I. FOOD AND WATER CONSUMPTION, BODY WEIGHT, SELECTED BLOOD CONSTITUENTS AND INSULIN:GLUCOSE RATIOS, 1 WEEK EXPERIMENT[a]

	Control	3.5% KOx	3.5% KOx + 2% uric acid
Body wt at sacrifice (g)	195.4 ± 3.3 (14)	177.5 ± 2.8[d] (14)	129.3 ± 1.8[d] (14)
Food consumption (5 day, g)	69.4 ± 1.1 (14)	52.6 ± 1.5[d] (14)	18.3 ± 1.6[d] (14)
Water consumption (7 day, ml)	130.0 ± 5.4 (12)	153.7 ± 4.5[d] (10)	144.7 ± 8.6[d] (12)
Uric acid[b] (mg/dl)	2.3 ± 0.2 (20)	4.6 ± 0.2[d] (18)	5.0 ± 0.3[d] (16)
Lactic acid[c] (mg/dl)	6.6 ± 0.3 (6)	13.8 ± 1.8[d] (4)	13.5 ± 1.4[d] (5)
Glucose (mg/dl)	144.0 ± 5.2 (14)	140.4 ± 4.5 (14)	179.1 ± 6.2[d] (14)
Insulin (μU/ml)	20.8 ± 3.1 (13)	19.1 ± 2.6 (12)	13.0 ± 1.1[d] (6)
Insulin:glucose (μU:ml/mg/dl)	0.142 ± 0.018 (13)	0.121 ± 0.009 (12)	0.072 ± 0.005[d] (6)

[a] Mean ± SEM, number of animals in brackets.
[b] Mean ± SEM of values from two separate experiments.
[c] These values from a separate 1 week feeding trial.
[d] Different from control, $P < 0.05$.

insulin by the β-cells of the pancreas (3) it can decrease renal clearance of UA (1) and thus might have contributed to the hyperuricemia.

Of particular interest was the observation that animals fed diets supplemented with UA had higher serum glucose values than controls. These elevated glucose levels were accompanied by lower insulin values compared to controls resulting in a significant decrease in the insulin:glucose ratio which can be taken as a sensitive indicator of abnormal insulin release (19, 20). Although decreased food intake by itself can lower serum IRI, serum glucose is not elevated in this circumstance (21). Thus, these animals exhibited several features seen in diabetes such as weight loss, polydipsia, polyuria (17), hypoinsulinemia, and hyperglycemia with reduced insulin:glucose ratios.

Figure 1 shows the effects of feeding KOx or KOx plus uric acid on serum IRI. When 3.5% KOx was fed for 1 week, serum IRI was not significantly altered. However, when the KOx diet was supplemented with 2% uric acid, serum IRI was reduced by 41%. Thus, it seems likely that levels of UA attained by feeding KOx alone for 1 week were insufficient to lower insulin output by the pancreas whereas UA supplementation of the diet markedly reduced serum IRI. Longer exposure to slightly higher concentrations of dietary KOx alone (4 weeks, 4.5%) increased serum UA 2.3-fold and decreased serum IRI from 19.7 ± 2.1 μU/ml to 14.5 ± 1.3 μU/ml (mean ± SEM, $P < 0.05$).

In interpreting results obtained using the KOx-treated rat it is important to differentiate the effects as being due to KOx, endogenous UA, or both. The decrease in serum IRI seen when the KOx diet was supplemented with UA suggests that indeed the effects we observe are due to UA or are UA induced and not due to KOx. To further distinguish these possibilities, we examined the effects of UA and KOx on the insulin secretory capacity of neonatal rat pancreatic islet cultures *in vitro*. In two separate experiments, each with three pancreatic cultures, 0.1 m*M* KOx did not influence insulin secretion by the cells (data not shown). However, exposing cultures to UA (0.04–0.5 m*M*) produced significant suppression of insulin secretion in six con-

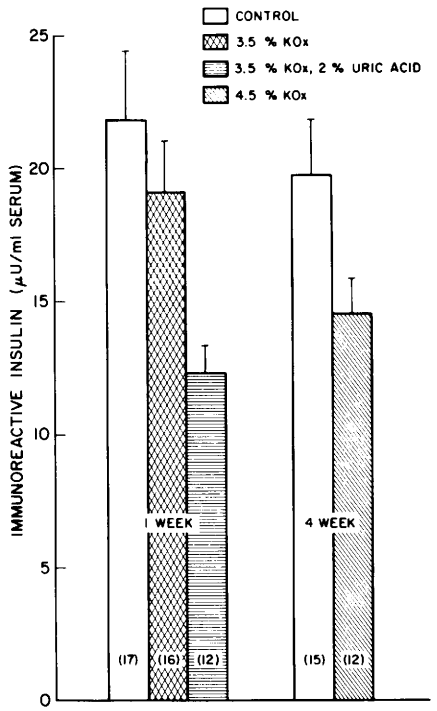

FIG. 1. Effects of dietary potassium oxonate and uric acid on rat insulin secretion. Male Wistar rats were fed a modified AIN-76 diet *ad libitum* containing 3.5% KOx and 3.5% KOx plus 2% UA for 1-week or 4.5% KOx for 4 weeks as indicated. Animals were fasted 16 hr overnight prior to exsanguination while under light ether anesthesia. Insulin was determined using the double-antibody technique of Hales and Randle (8). Values presented are the mean ± SEM from two separate 1-week experiments and two separate 4-week experiments. Numbers in parentheses indicate total number of animals.

secutive experiments. In the two experiments shown in Fig. 2, it can be seen that shortly after introduction of UA (0.04, 0.2 m*M*), the amount of insulin secreted by the cultures decreased significantly, reaching levels 35–70% of control values at 60 min. Since inhibition of insulin biosynthesis would only be apparent after 60 to 90 min (22), the rapidity of this response suggests an effect of UA on insulin secretion. An indication that the cells remained functionally intact after exposure to UA or KOx was confirmed by finding a brisk secretory response to 11.1 m*M* D-glucose 60 min later.

Discussion. Despite certain drawbacks associated with the KOx-fed rat as a model of hyperuricemia (reduced weight gain, possible inhibition of *de novo* purine and pyrimidine synthesis, see Refs. 6, 17, 18, 23–25, we have observed a decrease in serum IRI in animals fed KOx/UA-supplemented diets for 1 week with a similar effect seen in animals fed KOx-supplemented diets for 4 weeks. These data suggesting an effect of UA (direct or indirect) on serum IRI are further supported by the finding of a UA-induced decrease in insulin secretion by pancreatic islet cells in culture. Given the rapidity of the UA effect *in vitro*, we may speculate that the mechanism involved is related to interference with insulin secretion.

The controversy surrounding the relationship of uric acid to development or exacerbation of diabetes derives mainly from (i) inconsistent results from experiments using animals treated with UA alone (1–5) and (ii) an apparent lack of a higher incidence of diabetes in patients with gout (1, 2, 26). Our results using the uricase-inhibited rat and cultured pancreatic islets suggest that the failure of previous workers to observe a consistent decrease in serum insulin in animals treated with uric acid was probably due to the presence of a potent uricase degrading the UA. Moreover, emphasis in previous human studies has been on the concomitant occurrence of hyperuricemia and diabetes, a situation which may be relatively rare given the tendency toward uricosuria in uncontrolled diabetes (26, 27). Thus, in light of our results, the recent findings of Herman and co-workers (26) that prediabetic human males had higher serum UA levels than normal or diabetic subjects suggest that any contribution of UA to the development of diabetes would likely occur prior to the appearance of clinical symptoms (i.e., the relationship may be one of cause and effect).

However, it must be borne in mind that in addition to endogenous hyperuricemia associated with gout, there are several circumstances in which elevated UA levels may be encountered (1) such as consumption of certain foods (i.e., fructose (28,

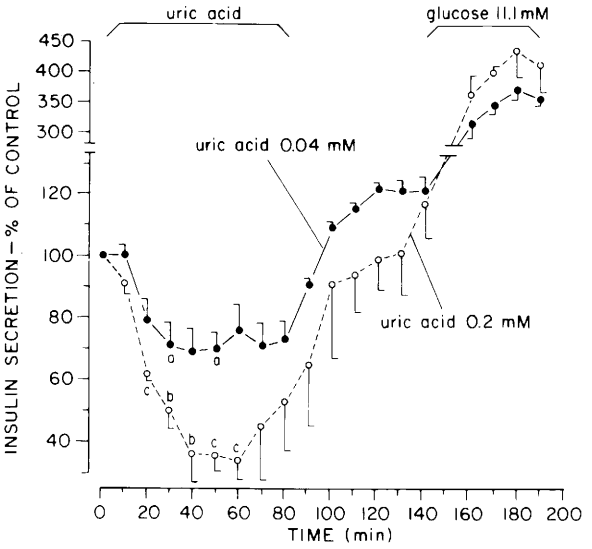

FIG. 2. Effect of uric acid on insulin secretion from cell cultures of neonatal rat pancreas. Following a 2 hr equilibration period, UA was added for 80 min. The UA-containing medium was then exchanged for fresh medium and 60 min later, the cultures were challenged with 11.1 m*M* D-glucose. The total insulin secreted during each 10-min period was expressed as a percentage of insulin secretion prior to addition of the test substance. Each point represents the mean ± SEM from one experiment with three culture flasks. Actual control insulin values for the 0.04 and 0.2 m*M* UA experiments were 4.6 ± 0.2 and 6.4 ± 1.0 ng/min/culture, respectively. Significance of the difference between values for the test period and baseline values was assessed using Student's *t* test. *P* values less than 0.05, 0.025, or 0.01 are represented by a, b, or c, respectively.

29), or purine-rich food) and drug treatment (i.e., diuretics or anti-cancer drugs (1)). Since relatively few individuals with hyperuricemia develop diabetes (possibly due to the presence of protective factors such as UA-binding proteins in blood (30)) elevated UA levels may be only one of several predisposing factors in the development of glucose intolerance and diabetes. Nonetheless, sustained or even transient hyperuricemia may be of greater concern as a risk factor for diabetes in certain genetically predisposed (31) or high-risk segments of the population such as obese or carbohydrate-sensitive individuals (20).

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