Factors in the Pathogenesis of Experimental Nonketotic and Ketoacidotic Diabetic Stupor

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SUMMARY

In an attempt to elucidate some of the factors responsible for the absence of significant ketosis in hyperosmolar nonketotic diabetic stupor, an experimental model was created in rats. This involved the intravenous administration of alloxan (40 mg. per kilogram) to produce moderate hyperglycemia, followed by daily hydrocortisone injections and dextrose feeding. For comparison, ketoacidotic diabetic rats and various control situations were also studied.

The biochemical profiles of the nonketotic and ketoacidotic diabetic rats resembled those of their clinical counterparts, although some discrepancies were apparent. Attention was directed at measuring plasma free fatty acid (FFA) and ketone body levels, as well as hepatic glycogen and portal and peripheral plasma immunoreactive insulin (IRI) concentrations in the diabetic and control groups. The hyperosmolar nonketotic diabetic rats were characterized by: a mean plasma FFA level intermediate between that of the controls and that of the ketotics; modest rises in 3-hydroxybutyrate and acetoacetate; and a striking elevation in hepatic glycogen concentration. In addition, their mean portal IRI value resembled that of the control animals (both significantly greater than ketotics) but their mean peripheral plasma IRI value was similar to that of the ketotics (both significantly lower than controls).

It is suggested that the absence of severe ketosis in the hyperosmolar nonketotic diabetic rats was, at least in part, due to the ability of their glycogen-rich, 'insulinized' livers to metabolize incoming FFA largely along nonketogenic pathways. The relevance of these findings to the clinical situation is briefly considered. DIABETES *22*:653-57, September, 1973.

The increasing frequency with which hyperosmolar nonketotic diabetic stupor is recognized in man has provoked much speculation about the pathogenesis of certain aspects of the syndrome. One of these concerns the absence of significant ketosis. A widely disseminated view postulates the suppression of lipolysis in adipose tissue—either because of small amounts of circulating endogenous insulin[1,2] or decreased levels of lipolytic hormones, combined with an effect of hyperosmolarity itself[3]—resulting in a decreased supply of free fatty acids (FFA) to the liver and consequent absence of hyperketonemia. On the other hand, a number of studies[4-6] have reported elevated, rather than suppressed, FFA levels in the acute phase of the syndrome, raising the possibility that antiketogenic hepatic factors may be operative. Because these cannot easily be studied in man, we have attempted to produce an animal model for nonketotic diabetic stupor. In this study we report our findings on the relationship between plasma FFA, ketone bodies, hepatic glycogen and portal and peripheral plasma immunoreactive insulin (IRI) concentrations.

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MATERIAL, EXPERIMENTAL DESIGN AND METHODS

Experimental design

Sprague-Dawley male rats, weighing between 250 and 350 gm., were chosen for the study.

In attempting to induce hyperosmolar nonketotic diabetes, the method suggested by Bavli and Gordon[7] was originally explored. This involved the production of moderately diabetic rats, who were then given hydrocortisone for four to six days and water deprived from the third day of steroid treatment. However, after several experiments yielded only a small number of biochemically suitable animals—and all of these with rather modest hyperglycemia and hyperosmolarity—the technic was modified. Alloxan (40 mg./kg.) was injected into the tail veins of lightly anesthetized rats; three days later, retro-orbital blood samples were measured for glucose concentrations and urine samples tested for glucose and ketones. Rats with blood sugars approaching 300 mg./100 ml. and aketotic glycosuria (generally about 50 per cent of the originally injected number) were selected for further study. This involved the administration of hydrocortisone (5 mg. intramuscularly daily) for an additional three-day period, on the last two days of which the drinking water was changed to 5 per cent dextrose. By the end of this time the rats were in an extremely dehydrated, lethargic state and were sacrificed. Nonketotic hyperosmolar diabetic stupor was considered to have been produced if subsequent analysis revealed severe hyperglycemia, in the absence (or at most a trace) of plasma ketosis as detected by Ketostix strips, a carbon dioxide content above 15 mEq./L. and a calculated serum osmolarity above 320 mosmol./L.

Ketoacidotic diabetic rats were produced by injecting a larger initial dose of alloxan (60 mg./kg.). They were sacrificed three days later, after urinalysis showed heavy glycosuria and ketonuria. Their preterminal clinical state was similar to that of the nonketotic diabetic animals.

Healthy, normally fed rats served as the control group. In order to assess the extent to which dextrose feeding, with or without hydrocortisone administration, was directly contributing to the biochemical findings observed in the nonketotic diabetic males, appropriate experimental situations were created. These included: healthy rats given 5 per cent dextrose water to drink for three days; healthy rats maintained on 5 per cent dextrose water and given hydrocortisone (5 mg. intramuscularly daily) for three days; rats injected intravenously with alloxan (40 mg./kg.) and, three days later, given 5 per cent dextrose to drink but no hydrocortisone; and rats injected intravenously with alloxan (60 mg./kg.) and immediately thereafter given 5 per cent dextrose water, together with daily hydrocortisone injections, for three days.

All groups of animals were kept on a standard laboratory chow, with food pellets available throughout the experiments. At the time of sacrifice, they were lightly anesthetized with ether and bled by aortic puncture. Simultaneous portal vein sampling was also performed. Small (50-100 mg.) amounts of liver tissue were removed for immediate weighing. Preterminal urine samples were tested for glucose and ketones.

Biochemical determinations

Serum urea and electrolytes were measured on aortic blood by standard microtechnics; total and direct serum bilirubin was also estimated, to assess the degree of hepatotoxicity produced by the acutely administered alloxan; plasma glucose was determined by a glucose oxidase method;[8] osmolarity was calculated from the preceding data;[3] ketones were assessed semiquantitatively in serum and urine with Ketostix strips (Ames); in a few animals actual levels of 3-hydroxybutyrate (3-OHBT) and acetoacetate were measured by an enzymatic technic;[9] FFA were determined on plasma samples (after blood for this purpose was placed in iced, heparinized tubes and the resultant plasma deep-frozen until analysis) by a modification of the Dole method.[10] Insulin was assayed in plasma samples taken from the aorta and also from the portal vein by a double antibody precipitation technic,[11] using a rat insulin standard curve. The glycogen concentration in samples of hepatic tissue was estimated colorimetrically;[12] not more than two minutes elapsed between removal of liver specimens and their immersion (after weighing) in methanol.

RESULTS

Blood glucose and electrolytes

Plasma glucose, serum urea and electrolyte compositions from healthy control, ketoacidotic and dextrose-fed hyperosmolar nonketotic diabetic rats are summarized in table 1. The nonketotic diabetic animals differed from their ketotic counterparts by showing: significantly higher ($p < 0.001$) serum sodium, chloride and carbon dioxide levels; significantly lower ($p < 0.001$), although elevated, urea values; and insignificantly greater glucose concentrations and calculated serum osmolarity. Not shown in the table are the results from our original series of nonketotic rats whose experimental protocol involved water deprivation and not dextrose water administration (seven in number); their plasma glucose averaged 248 (± 19) mg./100 ml. and serum osmolarity 329 (± 11) mosmol./L. Also not shown are the serum bilirubin measurements in the three groups. Both total and direct fractions were entirely

TABLE 1

Plasma glucose and serum urea and electrolyte values (mean ± SEM) in control, ketoacidotic and hyperosmolar nonketotic diabetic rats

Group	No. of rats	Plasma glucose (mg./100 ml.)	Serum sodium (mEq./L.)	Chloride (mEq./L.)	Potassium (mEq./L.)	Carbon dioxide content (mEq./L.)	Urea (mg./100 ml.)	Osmolarity (mosmol./L.)
Controls	10	94 ± 3	142 ± 2	107 ± 1	4.9 ± 0.1	16 ± 1	50 ± 2	308 ± 3
Ketotic	10	724 ± 61	119 ± 2	77 ± 3	6.1 ± 0.6	9 ± 1	250 ± 22	333 ± 4
Hyperosmolar nonketotic	8	766 ± 25	134 ± 2	99 ± 2	5.9 ± 0.4	17 ± 1	118 ± 16	343 ± 6

normal (< 0.3 mg./100 ml. and < 0.2 mg./100 ml. respectively) in all instances, implying an absence of serious hepatoxicity in the alloxan-treated animals.

Plasma FFA, ketone body and hepatic glycogen concentrations

Table 2 outlines the plasma FFA and actual ketone body levels, as well as the hepatic glycogen concentrations, in the three groups whose biochemical details were given in table 1. The nonketotic diabetic rats were characterized by: a mean FFA value that was significantly greater ($p < 0.01$) than that of the controls but significantly lower ($p < 0.01$) than the mean of the ketotics; modest rises in plasma 3-OHBT and acetoacetate levels compared with controls ($p < 0.05$ for both) although they were well below ($p < 0.01$ for both) concentrations observed in the ketotic animals; and striking elevations in hepatic glycogen concentrations ($p < 0.02$ versus controls and $p < 0.001$ versus ketotics).

It is noteworthy that *individual* FFA levels among the nonketotic and ketotic diabetic groups showed some overlap, although the highest concentrations (above 2,000 μEq./L.) were found only in the ketotic rats. Of interest, too, was the observation that mean liver glycogen was also elevated—at 3.59 (± 0.38) mg./100 mg. wet weight—in our initial nonketotic moderately diabetic rats subjected to water deprivation but not fed dextrose.

Portal and peripheral plasma IRI responses

These are shown in table 3. With respect to portal vein IRI, the hyperosmolar nonketotic diabetic rats resembled the control animals, the mean level in each group being significantly higher ($p < 0.01$) than that of the ketotic group. However, with respect to peripheral (i.e., aortic) plasma IRI measurements, the nonketotic diabetic rats were similar to the ketotic animals, the mean value in each group being significantly lower ($p < 0.05$) than that of the controls.

Various 'control' situations

In none of the other experimental situations where the animals were given 5 per cent dextrose water to drink, with or without hydrocortisone, was the hyperosmolar nonketotic syndrome produced (table 4). Noteworthy findings, however, were a substantial elevation of hepatic glycogen after dextrose feeding alone in healthy rats and an inability of the combined dextrose/hydrocortisone regime to increase hepatic glycogen stores (or prevent ketosis) when administered to animals given a 'ketotic' dose of alloxan.

Dextrose water alone, without concomitant hydrocortisone, was unable to induce the hyperosmolar syndrome in rats given the smaller (i.e., 40 mg./kg.) alloxan injection, implying an effect of hydrocortisone in augmenting hyperglycemia, osmolarity and hepatic glycogenesis in these animals (although not in controls).

DISCUSSION

Antiketogenesis in the liver depends on several factors,[13] one of which is related to the availability of carbohydrate, in the form of hepatic glycogen stores.[14]

TABLE 2

Plasma FFA, ketone body and hepatic glycogen concentrations (mean ± SEM) in control, ketoacidotic and hyperosmolar nonketotic diabetic rats

Group	Plasma FFA (μEq./L.)	Plasma ketone bodies (mg./100 ml.)		Hepatic glycogen (mg./100 mg. wet weight)
		3-OHBT	Acetoacetate	
Controls	807 ± 35(6)	1.09 ± 0.17	0.23 ± 0.04(6)	2.68 ± 0.32(10)
Ketotic	2,035 ± 207(8)	48.46 ± 4.91	19.65 ± 3.94(6)	0.64 ± 0.11(10)
Hyperosmolar nonketotic	1,274 ± 124(8)	12.90 ± 5.40	4.37 ± 1.24(6)	4.15 ± 0.45(8)

The number in parentheses indicates the number of animals per investigation.

TABLE 3
Portal vein and peripheral plasma IRI responses (mean ± SEM) in control, ketoacidotic and hyperosmolar nonketotic diabetic rats

Group	No. of rats	Portal vein IRI (μU./ml.)	Peripheral plasma IRI (μU./ml.)
Controls	8	35 ± 4	28 ± 3
Ketotic	5	13 ± 1	13 ± 1
Hyperosmolar nonketotic	6	33 ± 5	18 ± 4

Findings in the present study indicate striking differences in liver glycogen concentrations between ketotic and nonketotic diabetic animals. The absence of substantial ketosis in the latter group may, therefore, in part be due to the fact that their incoming FFA are consequently being predominantly, although not completely, metabolized along nonketogenic pathways—i.e., by esterification into triglyceride and oxidation via the citric-acid cycle to carbon dioxide. The comparatively lower level of FFA in the nonketotic diabetic rats probably also contributed to the lack of appreciable ketosis[14] but, since they were still considerably raised (particularly in relation to the prevailing blood glucose concentrations) and in some cases overlapped with values in the ketotic group, it seems unlikely that this factor *alone* can account for it. Reasons for the dissimilar mean FFA levels in the nonketotic and ketotic diabetic rats are, in fact, unclear, particularly as there was no significant difference in peripheral plasma IRI concentrations between the two groups. It might, however, reflect an enhanced biological activity of insulin in the hyperosmolar nonketotic syndrome, unopposed by antagonists which are usually present in the ketotic state, namely severe acidosis together with elevated growth hormone[3,15] and pancreatic glucagon[16] values.

Why were hepatic glycogen levels so high in the experimental hyperosmolar nonketotic syndrome? It seems reasonable to attribute this finding mainly to the difference in *portal* vein insulin concentrations between the nonketotic and ketotic animals. The former showed similar portal IRI levels to the controls, although this probably represented their maximal beta cell response to extreme hyperglycemia. In the presence of sufficient insulin to maintain key liver enzymes in a normal state, excess glycogen would be synthesized when both glucose and hydrocortisone were subsequently administered. In our original nonketotic moderately diabetic rats whose liver glycogens were raised despite not being given dextrose water, endogenous hyperglycemia—together with the hydrocortisone injections—might have been the glycogenic stimulus. This concept of an 'insulinized liver but diabetic periphery' was originally advanced by Haft,[17] to explain the hepatic hyperglycogenesis found in insulin treated, alloxan-diabetic rats who still had marked hyperglycemia.

Arising from this is the interpretation of the varying portal:peripheral IRI ratios in the three groups of rats. In control animals the mean portal IRI concentration was only slightly above the peripheral value, not unlike the situation in fasting nondiabetic humans.[18] The marked fall off in peripheral IRI concentrations in the hyperosmolar nonketotic rats suggests that substantial trapping or utilization of insulin occurred in their livers, while the identical, very low, portal and peripheral vein levels in the ketotic group could reflect the failure of these hepatic functions and/or the reduced activity of insulin in the ketoacidotic milieu. Further studies in this direction seem warranted.

TABLE 4
Pertinent biochemical findings (mean ± SEM) in rats given dextrose water with or without hydrocortisone injections for three days

Group	No. of rats	Plasma glucose (mg./100 ml.)	Serum osmolarity (mosmol./L.)	Hepatic glycogen (mg./100 mg. wet weight)
5% dextrose water alone	6	87 ± 5	307 ± 1	4.18 ± 0.35
Dextrose water and hydrocortisone injections	7	95 ± 13	308 ± 4	2.35 ± 0.33
Dextrose water with 'nonketotic' dose of alloxan	5	299 ± 103	303 ± 12	1.36 ± 0.24
Dextrose water and hydrocortisone with 'ketotic' dose of alloxan	6	385 ± 36*	333 ± 8	0.82 ± 0.42

* Associated with severe ketoacidosis

Finally, the relevance of the experimental nonketotic model to the pathophysiology of clinical hyperosmolar nonketotic diabetic stupor is of interest. Biochemically the animal model resembles its clinical counterpart in many respects,[19] although serum sodium, osmolarity and urea values appear to be somewhat lower in the rats. (The disproportionately elevated urea levels found in the ketotic animals may have been partly due to a dose-related alloxan-induced nephrotoxicity.[20]) In addition, there are reports suggesting that provocative factors similar to the experimental ones are operative in some clinical situations. Thus excessive carbohydrate consumption,[19,21,22] hypertonic glucose solutions in peritoneal dialysis[23] and pharmacological doses of corticosteroids[24] appear to have triggered the syndrome in mildly diabetic patients. In these situations, at least, the occurrence of elevated hepatic glycogen could similarly be anticipated, thereby contributing to the absence of significant ketosis at varying FFA concentrations. The occasional documentation[25,26] of raised hepatic glycogen levels also occurring in brittle, ketosis-prone diabetics might, therefore, seem antithetic, but as these patients were either pretreated with insulin or only mildly ketotic, they are probably not comparable with acute, untreated ketoacidotic diabetics in whom depleted liver glycogen has certainly been demonstrated.[27]

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