Increased Production of Fructose by the Brain in Dogs Made Diabetic by Alloxan and by Growth Hormone

Bruce S. Spinowitz, M.D., and Norman Altszuler, Ph.D., New York

SUMMARY

The cerebrospinal fluid (CSF) concentration of fructose is elevated in diabetes. In the alloxan-diabetic animal this elevation has been attributed to increased fructose production by the brain. The present study seeks to determine if the increased fructose production is due to hyperglycemia or insulin deficiency. C-14-fructose was injected into the CSF in anesthetized dogs, and from its rate of removal the turnover of CSF fructose was calculated. Since plasma fructose does not pass readily into CSF, the fructose flux reflects fructose production by brain.

In dogs made diabetic with growth hormone, which results in hyperglycemia and hyperinsulinism, CSF fructose levels and turnover rate are significantly elevated. Restoration of normoglycemia by a thirty-six hour fast returns CSF fructose levels to normal. Glucose infusion to elevate plasma glucose for four hours in normal dogs raises plasma fructose but not CSF fructose levels. Similarly, eliminating hyperglycemia for three hours in alloxan-diabetic dogs by insulin infusion decreases plasma fructose, but CSF fructose levels remain elevated. Daily insulin injections do restore CSF fructose to normal.

It appears that increased fructose production by the brain in diabetes depends on hyperglycemia and the underlying metabolic processes for this require a number of hours to develop or be expressed. DIABETES 22:820-24, November, 1973.

Significant concentrations of fructose in the cerebrospinal fluid (CSF) in normal animals was first demonstrated by Hubbard and Russell using resorcinol.[1] The findings were confirmed almost thirty years later by Wray and Winegrad, who used a more sensitive enzymatic assay.[2,3] Since it was shown that D-fructose does not cross the blood-brain barrier to any measurable extent,[4-6] these investigators postulated that elevated CSF fructose levels with diabetes resulted from increased fructose production by brain tissue, which had been shown to have the enzymes necessary for conversion of glucose to fructose via sorbitol.[7,8] The linear relationship between concentrations of CSF glucose and CSF fructose tended to support this hypothesis.[2] Margolis et al. studied the flux of fructose in the CSF of alloxan-diabetic dogs and demonstrated that elevated CSF fructose concentration was indeed due to increased fructose formation by the brain.[9]

The present studies were undertaken to determine if the increased CSF fructose in diabetic animals is due to a deficiency of insulin or to concomitant hyperglycemia.

MATERIALS AND METHODS

Experiments were performed on male and female dogs (10 to 15 kg.), fasted for eighteen to twenty hours and anesthetized with sodium pentobarbital (30 mg. per kilogram). Diabetes was produced by the injection of alloxan, 50 mg. per kilogram intravenously, or by a regimen of bovine growth hormone, 3 mg. per kilogram per day for six to eight days. Only dogs with a plasma glucose concentration over 180 mg./100 ml. were accepted as having diabetes. Blood samples were taken from the jugular vein through an indwelling polyethylene catheter, and CSF was withdrawn from the cisterna magna through a spinal needle left in place during the course of the experiment. All of the CSF samples were clear of blood and turbidity.

Samples of 0.1 ml. of CSF and plasma were deproteinized with two or five volumes, respectively, of 0.33 M. perchloric acid. The concentration of glucose and fructose in plasma and cerebrospinal fluid was measured enzymatically after phosphorylation with yeast hexokinase and oxidation with glucose-6-phosphate dehydrogenase (in the presence of NAD), before and after addition of phosphoglycoseisomerase.[8]

To determine the rate of efflux of fructose from the CSF, C-14-fructose, in weightless amount, was injected

From the Department of Pharmacology, New York University School of Medicine, New York, New York 10016.

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into the cisterna magna in a volume of 0.5 ml. of CSF. Samples of CSF were taken at half-hour intervals for three hours, and the radioactivity was measured by liquid scintillation. The half-time of fructose efflux was calculated from the slope of a semilogarithmic graph of the disappearance of radioactivity. These data, along with the measured concentration of fructose, were used to calculate the production of fructose by the brain, as shown in the following formula:

$$\text{turnover rate (mg./hr.)} = \frac{\text{pool size}}{(1.44)\ (T_1/_2 \text{ of efflux})}$$

The pool size of fructose in CSF is calculated from the CSF concentration of fructose, multiplied by the total volume of the CSF, estimated to be 12 ml. in these dogs. In the steady state fractional turnover rate is a measure of inflow of fructose to CSF and of outflow of fructose from CSF. Since studies have demonstrated that plasma fructose does not pass readily into the CSF,[9] even when plasma levels are elevated significantly, the inflow of fructose into the CSF, as measured here, represents fructose production by the brain.

RESULTS

The effects of alloxan diabetes on the flux of fructose in the CSF are shown in table 1. The plasma and CSF glucose concentrations are elevated as expected. Plasma and CSF fructose levels are likewise markedly elevated. The possibility that the elevated CSF fructose level might be due to a slower rate of fructose efflux from the CSF can be ruled out, since, as seen in table 1, the rate of fructose efflux from the CSF is nearly tenfold greater in dogs with alloxan diabetes (1.63 mg. per hour) than in normal dogs (0.18).

The effect of diabetes induced by growth hormone on the above parameters was then investigated because this type of diabetes is characterized by very high levels of plasma insulin.[10,11] As seen in table 1, plasma and CSF glucose concentrations are elevated to 200 and 145 mg./100 ml., respectively, indicating diabetes. Although the plasma fructose concentration is only slightly above normal, the CSF fructose level is markedly elevated to 11.3 mg./100 ml. While the half-time of fructose efflux in these animals is not different from normal, the rate of CSF fructose inflow and outflow is significantly elevated, indicating that the elevated CSF fructose concentration is due to increased fructose formation in the brain rather than decreased efflux from the CSF.

Animals with diabetes induced with growth hormone were used to explore the role of hyperglycemia and insulin deficiency as factors contributing to the elevation of fructose in the CSF. Fasting prevents the growth hormone-induced increase in plasma glucose concentration and in glucose turnover, but does not prevent a rise in plasma insulin;[10] the fast lowers the elevated plasma glucose levels, while the insulin levels still remain significantly above normal values (unpublished observations). This approach was utilized to assess the role of hyperglycemia in dogs with diabetes induced with growth hormone. The effect of a thirty-six hour fast on fructose flux is shown in table 2. Fasting resulted in the return to near normal of the plasma and CSF glu-

TABLE 1

Effect of diabetes induced with alloxan or growth hormone on CSF fructose concentration and turnover in the dog

Measurements	Normal dogs (6)*	Alloxan diabetes (9)	Growth hormone† diabetes (4)
Plasma glucose concentration (mg./100 ml.)	80 ± 5‡	276 ± 20	200 ± 21
CSF glucose concentration (mg./100 ml.)	63 ± 4	154 ± 4	145 ± 11
Plasma fructose concentration (mg./100 ml.)	1	5.0 ± 0.9	1.2 ±0.7
CSF fructose concentration (mg./100 ml.)	1	7.6 ± 0.3	11.3 ± 2.5
$T_{1/2}$ fructose efflux from CSF (min.)	51 ± 2	39 ± 2§	57 ± 2
CSF fructose turnover (mg./hr.)	0.09 ± 0.01	0.97 ± 0.08‖	1.00 ± 0.32‖
Fructose production by brain (mg./kg./hr.)	1.2	12.9	13.3

* Number of experiments.
† Bovine growth hormone (NIH-GH-B16), 3 mg. per kilogram per day for six to eight days.
‡ Mean ± standard error of mean.
§ Significantly different from normal, $P < 0.05$.
‖ Significantly different from normal, $P < 0.01$.
Fructose production calculated by multiplying turnover rate in CSF by 13.3 (based on 75 gm. as an average dog brain weight).

TABLE 2

Plasma and CSF concentrations of glucose and fructose in dogs with diabetes induced with growth hormone in postabsorptive and fasting states

Measurements	Growth hormone* diabetes (3)†	Growth hormone diabetes + fasting‡ (3)
Plasma glucose concentration (mg./100 ml.)	199 ± 21	98 ± 1
CSF glucose concentration (mg./100 ml.)	145 ± 11	54 ± 3
Plasma fructose concentration (mg./100 ml.)	1.2 ± 0.7	1
CSF fructose concentration (mg./100 ml.)	11.3 ± 2.5	2.2 ± 0.9

* Bovine growth hormone (NIH-GH-B16), 3 mg. per kilogram per day, six to eight days.
† Number of experiments.
‡ Fasted for thirty-six hours, with continuation of growth hormone administration.

cose concentrations. CSF fructose concentration has also fallen to a value not significantly different from normal, 2.2 mg./100 ml. Thus, eliminating hyperglycemia only, while plasma insulin levels are still above normal, results in a lowering of the CSF fructose concentration to normal.

The effect of hyperglycemia on CSF fructose was also studied in normal dogs. Figure 1 shows the effects of a glucose infusion, 1 to 1.5 gm. per hour, on the levels of fructose in plasma and CSF. Elevation of glucose levels to those seen in the diabetic animal fails to cause a rise in CSF fructose levels during the four hour period of observation. Since the plasma fructose levels during this period rose significantly, this lack of change in the CSF fructose level is further evidence that plasma fructose does not pass readily into the brain. The present finding—that acute, transient hyperglycemia does not raise CSF fructose—is in disagreement with the findings of Clements et al., who observed an elevation of CSF fructose to 6 mg./100 ml. after a four hour infusion of glucose. However, larger doses of glucose, 3 to 4 gm. per kilogram per hour, were used, which elevated plasma glucose levels to 500 mg./100 ml. and created hyperosmolar conditions. The conditions of the present studies more closely resemble the transient mild elevation seen when diabetes is slightly out of control. The observed difference may also be due to the kinetic characteristics of aldose reductase, which is the rate-limiting enzyme of the sorbitol pathway. Since the Km for this enzyme is high, the low CSF glucose concentration attained in the present experiments would tend to delay the conversion of glucose to fructose.

In another approach to determine the effect of hyperglycemia on CSF fructose, plasma glucose levels were lowered in the alloxan diabetic by a prolonged infusion of insulin. The insulin infusion was decreased in stepwise fashion during the first forty minutes: 0.2 U. per kilogram per hour was infused for twenty minutes and 0.1 U. per kilogram per hour for the next twenty minutes, and the infusion was then continued for five hours at 0.05 U. per kilogram per hour. The results are shown in figure 2. Insulin caused a rapid fall in plasma glucose levels so that the animal was normoglycemic for the last three hours of the six hour infusion. Despite the normoglycemia, CSF fructose concentration remained signifi-

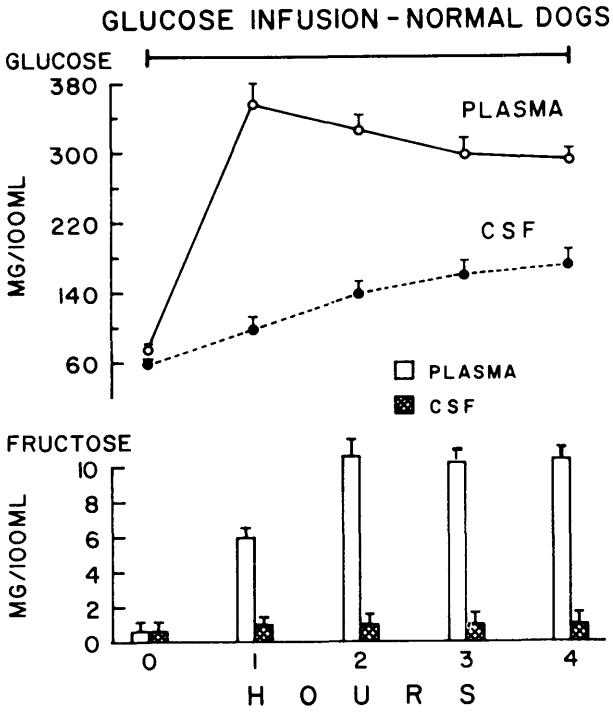

FIG. 1. Plasma and CSF concentrations of glucose and fructose in normal dogs during continuous infusion of glucose at 1 to 1.5 gm. per hour for four hours. Glucose concentration increased in plasma and CSF, while fructose concentration increased in plasma but not in CSF. Vertical lines represent 2 x standard error of mean.

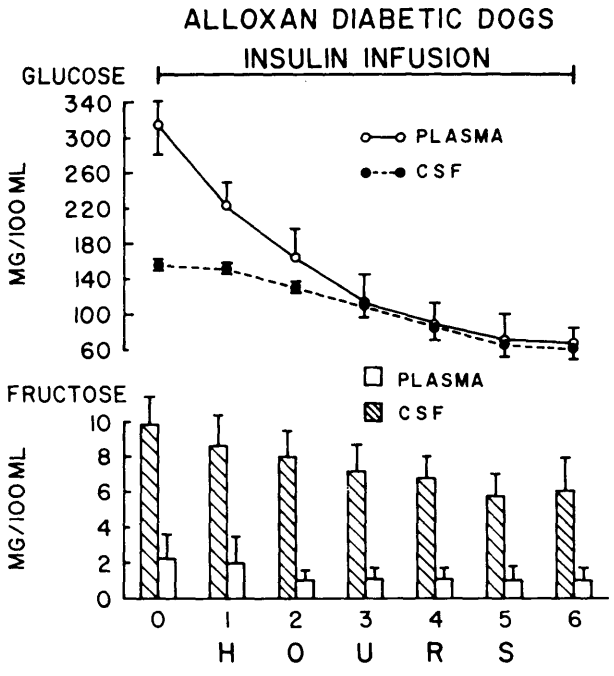

FIG. 2. Plasma and CSF concentrations of glucose and fructose in alloxan-diabetic dogs. Insulin was infused in decreasing amounts during first forty minutes: zero to twenty minutes at 0.2 U. per kilogram per hour, twenty to forty minutes at 0.1 U. per kilogram per hour and at 0.05 U. per kilogram per hour for the remainder of six hour infusion. Plasma and CSF concentrations of glucose fell promptly and were at normal levels for last three hours, while CSF fructose remained significantly above normal levels.

cantly higher than normal, 6 mg./100 ml. as compared with 1 mg./100 ml. Thus, CSF fructose production appears to remain above normal despite the removal of the hyperglycemic stimulus during the three hour period.

Long-term treatment of the alloxan-diabetic dog with insulin does lower the elevated CSF fructose levels. Table 3 shows the changes in the alloxan-diabetic dog maintained on daily doses of Protamine Zinc insulin, 0.8 to 1.2 U., for two weeks. The plasma and CSF glucose concentrations in the postabsorptive state are lowered to 81 and 52 mg./100 ml., respectively, and the plasma and CSF fructose concentrations, and presumably fructose production, are likewise lowered to normal levels.

DISCUSSION

The present finding is in agreement with the earlier report that fructose production by the brain is increased in alloxan diabetes.[9] Since this type of diabetes is characterized by both hyperglycemia and insulin deficiency, the relative role of each in the increased formation of CSF fructose remained to be determined. Several approaches were utilized, and the basis for each is discussed below.

The effect of chronic hyperglycemia without insulin deficiency was studied utilizing dogs made diabetic by a growth hormone regimen. This type of diabetes is characterized by hyperglycemia and greatly elevated, rather than decreased, concentrations of plasma insulin.[10,11] In these animals fructose production by the brain was markedly increased, as with alloxan diabetes, indicating that insulin deficiency is not essential for increased CSF production; on the other hand, after a brief fast, which resulted in normoglycemia with plasma insulin levels still elevated, CSF fructose levels returned to normal. Thus, in the growth hormone-treated animal increased fructose production by the brain and elevated CSF fructose levels occur only in the presence of hyperglycemia.

In another approach glucose was infused for several hours into normal animals. While this produces immediate hyperglycemia and elevates plasma insulin levels, only the plasma fructose concentration rose and the CSF fructose remained unchanged. Since hyperglycemia and elevated plasma fructose levels were maintained for three hours, it would appear that increased fructose formation by the brain requires a number of hours to develop. In part, this may be due to the kinetic characteristics of the aldose reductase, as discussed in the preceding section.

The time dependence for increased fructose formation by the brain is also evident from the experiments in the alloxan-diabetic animal. Here, infusion of insulin in appropriate dosage for six hours promptly lowered elevated plasma glucose and fructose levels, but CSF fructose concentration remained elevated. On the other hand, long-term administration of insulin, i.e., daily injections of Protamine Zinc insulin to maintain normoglycemia, did indeed lower CSF fructose levels to normal.

There have been recent suggestions that increased sorbitol, as well as fructose, concentrations in nervous tissue may be a factor in the various neuropathies seen with diabetes of long standing.[13] A more immediate consequence of elevated fructose levels with diabetes relates to the finding of cerebral edema at postmortem examination of subjects dying in diabetic coma.[14-16] It has been postulated that in the diabetic state prior to insulin treatment hyperglycemia would lead to increased fructose concentration in brain cells.[12,17] Dehydration and plasma hypertonicity, which are common in the ketoacidotic state, would tend to alleviate the conse-

TABLE 3

Plasma and CSF concentrations of glucose and fructose in alloxan-diabetic dogs before and during insulin therapy

Measurements	Alloxan diabetes (3)*	Alloxan diabetes + insulin Rx† (3)
Plasma glucose concentration (mg./100 ml.)	276 ± 20	81 ± 20
CSF glucose concentration (mg./100 ml.)	154 ± 4	52 ± 18
Plasma fructose concentration (mg./100 ml.)	5.0 ± 0.9	1
CSF fructose concentration (mg./100 ml.)	7.6 ± 0.3	1.3 ± 0.4

* Number of experiments.
† Maintained on Protamine Zinc insulin (0.8 to 1.2 U. per day per dog) for two weeks.

quences of increased fructose concentration in the brain. An abrupt decrease in plasma glucose and osmolality due to overzealous insulin therapy and rehydration might result in a sudden shift of water into the brain.[12,17] The same potential for cerebral edema could apply to excessive insulin therapy in hyperosmolar, nonketotic diabetic coma.[18] An alternative postulation, based on the data in figure 2, is that glucose disappears from the CSF at a slower rate than from the plasma. The disparity in the rates could serve as an osmotic stimulus, resulting in cerebral edema after rapid correction of blood glucose in diabetic coma.

The present findings that elevated CSF fructose levels depend on the presence of hyperglycemia, and that the abatement of the increased conversion of glucose to fructose requires many hours, reinforce the need for caution in correcting plasma hypertonicity in ketotic and nonketotic diabetic coma.[19]

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