On the Mechanism of Diazoxide-induced Hyperglycemia

Norman Altszuler, Ph.D., Jennifer Hampshire, Ph.D., and Ety Moraru, M.S., New York

SUMMARY

Infusion of diazoxide (16.5 mg./kg. in 10 minutes) into normal unanesthetized dogs resulted in a prompt hyperglycemia due to increased hepatic glucose production as measured with a 3-^{3}H-glucose primer-infusion technique. Plasma insulin and glucagon were decreased. Glucose uptake failed to increase. Diazoxide administration during period of alpha adrenergic receptor blockade with phentolamine still caused hyperglycemia and increased glucose production. Glucose uptake was inhibited despite adequate plasma insulin. Infusion of somatostatin along with insulin prevented the effects of diazoxide on plasma glucose and glucose production.

It is concluded that diazoxide hyperglycemia is not due solely to decreased insulin secretion or increased epinephrine secretion and that glucagon is not a contributory factor. Diazoxide may act directly to increase glucose production and inhibit glucose uptake. Somatostatin appears capable of blocking the effect of diazoxide on glucose production by an unknown mechanism. DIABETES *26*:931-35, October, 1977.

Diazoxide is a nondiuretic benzothiadiazine that has been shown to have a marked antihypertensive effect. It also produces a significant hyperglycemia, and its possible use in treatment of hypoglycemia has been the subject of a recent symposium.[1] The mechanism of the hyperglycemic effect of diazoxide was explored extensively, and these findings were also cited in the symposium.[1] Although decreased insulin secretion and increased epinephrine secretion have been implicated, the mechanism of the induced hyperglycemia remains undetermined.

The present study reëxamines the suggested explanations and also explores the role of glucagon in this regard.

Presented at the 36th Annual Meeting of the American Diabetes Association, San Francisco, June 20-22, 1976.

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

Accepted for publication June 19, 1977.

METHODS

Experiments were carried out on trained unanesthetized normal dogs weighing 16-20 kg. at about 18 hours after their daily meal. The animals were maintained on a standard diet, with the following source of calories: 38 per cent from carbohydrates, 39 per cent from protein, and 23 per cent from fat.[2] Glucose production and uptake were measured with 3-^{3}H-glucose, administered as a priming injection along with a constant infusion into a saphenous vein.[3] All other infusions were also in the saphenous vein. Serial blood samples were collected in heparinized syringes through an indwelling polyethylene tubing inserted percutaneously into the jugular vein shortly prior to start of experiment. The blood was promptly centrifuged and the plasma deproteinized with $Ba(OH)_2$ and $ZnSO_4$ according to the method of Somogyi.[4] Aliquots of plasma were frozen for analysis of insulin. For glucagon analysis, 4 ml. of blood was placed in chilled tubes containing 0.2 ml. of Traysylol and 0.2 ml. of 2.4 per cent Na_2EDTA (1.2 mg./ml. blood). The blood was centrifuged and plasma frozen for later analysis.

Plasma glucose was analyzed by the glucose oxidase method[5] on a Beckman Glucose Analyzer. Plasma insulin was determined by the radioimmunoassay method of Hales and Randle[6] with a kit from Schwartz-Mann, Orangeburg, N.Y. Glucagon was determined by the radioimmunoassay method of Faloona and Unger[7] with antiserum 30K obtained from Dr. Roger Unger and ^{125}I-glucagon from Nuclear Medical Laboratories, Dallas, Texas. Diazoxide was kindly donated by Dr. S. Symchowicz, Schering Corp., Bloomfield, N.J., phentolamine by Dr. A. J. Plummer, Ciba-Geigy Corp., Summit, N. J., and crystalline insulin glucagon by Dr. Mary Root, Eli Lilly and Co., Indianapolis, Ind.

Calculation of plasma glucose specific activity has been described in detail previously.[3] Rates of glucose production and over-all glucose uptake by tissue were calculated for the steady state as described before.[3,8] During periods of changing plasma glucose concentration, 0.7 of the initial glucose pool size was used as the rapidly mixing compartment for calculation of glucose production and uptake.[9-11]

RESULTS

The effect of infusion of diazoxide (16.5 mg./kg. in 10 minutes) into normal dogs is shown in figure 1. There is a prompt increase in plasma glucose concentration, which persists for at least 90 minutes. The plasma insulin concentration is decreased. The initial rise in plasma glucose is accompanied by an increase in glucose production. The increase is transient, and at 60 minutes glucose production is back to normal; hyperglycemia persists beyond that period presumably because of a lack of increase in glucose utilization. Indeed, glucose utlization fails to increase throughout the 90-minute period despite presence of hyperglycemia. The plasma insulin levels are decreased, but this may not explain the lack of increase in glucose uptake. It is evident that the hyperglycemia is not due to increased glucagon secretion and, in fact, plasma glucagon levels are decreased.

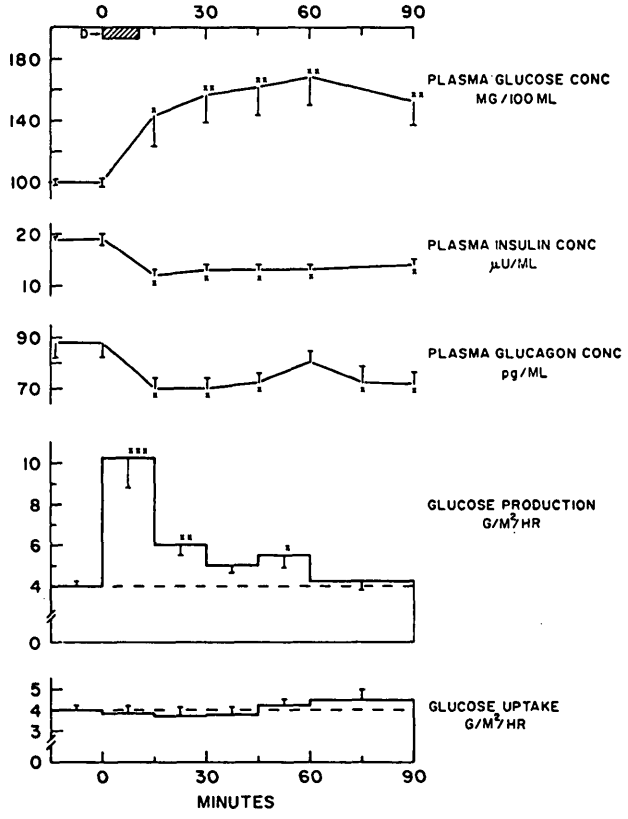

FIG. 1. Infusion of diazoxide, D, at 16.5 mg./kg. in 10 minutes, in six normal dogs caused a prompt hyperglycemia that persisted for the 90-minute period of observation. Plasma insulin and glucagon levels were decreased. Glucose production was increased promptly and significantly above the control values obtained in the three-hour period immediately before 0 time. Increased glucose production resulted in elevated plasma glucose values, but glucose uptake failed to increase despite the hyperglycemia. Statistical significance denoted as follows: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

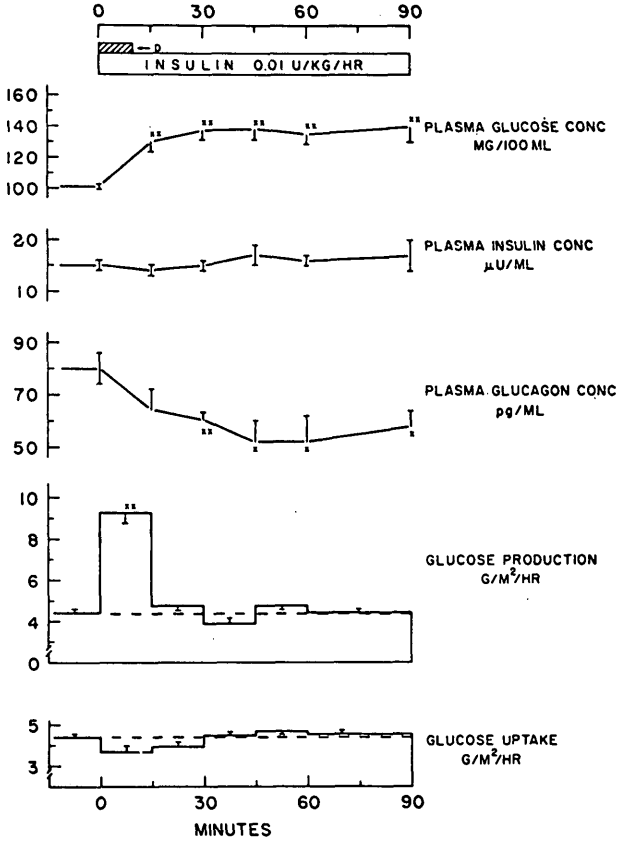

FIG. 2. Infusion of diazoxide (16.5 mg./kg. in 10 minutes superimposed on infusion of insulin (0.01 U./kg./min.) caused a prompt hyperglycemia and a fall in plasma glucagon levels. Glucose production was increased, resulting in the hyperglycemia; glucose uptake remained unchanged despite hyperglycemia and normal plasma insulin levels, indicating a relative inhibition of glucose uptake. Statistical designations as in figure 1.

The role of insulin deficiency in the hyperglycemic response to diazoxide was examined by replacement of insulin by a constant infusion. As seen in figure 2, diazoxide injection still results in hyperglycemia even with normal plasma insulin levels. Plasma glucagon

levels are again depressed. Glucose production is increased for a brief period of time, leading to the hyperglycemia. Glucose uptake is not increased despite hyperglycemia and normal insulin levels indicating inhibition of glucose uptake. When uptake is related to plasma glucose (i.e., metabolic clearance) it is seen that insulin did cause some increase in glucose uptake when compared with uptake with diazoxide alone (figure 1), but the net effect is inhibition of uptake.

The possibility that the diazoxide-induced hyperglycemia might be due to increased sympathetic activity was explored by the use of the alpha adrenergic receptor blocking agent phentolamine. As seen in figure 3, infusion of phentolamine, at a rate that blocks the hyperglycemia produced by epinephrine infusion at 0.2 μg./kg./min., fails to prevent the diazoxide-induced hyperglycemia. Plasma glucagor

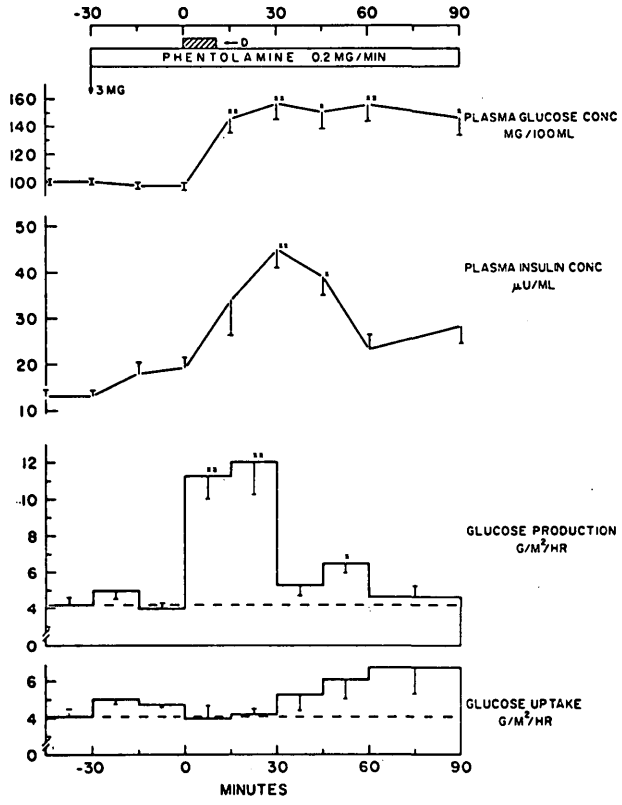

FIG. 3. Effect of pretreatment with phentolamine (3 mg. I.V. and infusion at 0.2 mg./min.) on the response to diazoxide (16.5 mg./kg. in 10 minutes) in five normal dogs. Phentolamine alone had no significant effects on the above measurements. Diazoxide caused a hyperglycemia, associated with increased glucose production. Glucose uptake was not significantly increased despite elevated plasma insulin values indicating resistance to insulin. Statistical designations as in figure 1.

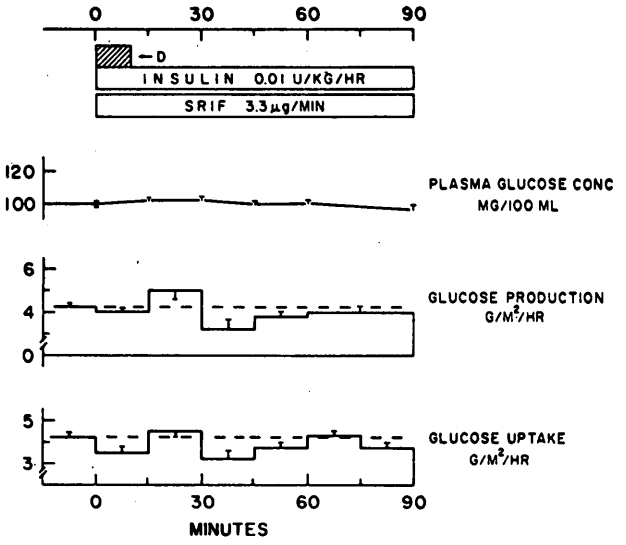

FIG. 4. Effect of combined infusion of somatostatin (SRIF; 3.3 μg./min.) and insulin (0.01 U./kg./min.) on the response to diazoxide (16.5 mg./kg. in 10 minutes) in five normal dogs. Diazoxide failed to increase glucose production or cause hyperglycemia.

values (not shown) did not increase. Plasma insulin shows a transient increase in response to the hyperglycemia, but glucose uptake fails to increase, indicating an impairment of glucose utilization. During the second hour (from 30 to 90 minutes) of phentolamine infusion the changes in glucose uptake are similar to those seen with phentolamine alone and thus are unrelated to the diazoxide administration.

To further examine the role of glucagon in the hyperglycemia response to diazoxide, somatostatin was infused but insulin deficiency was prevented by infusion of insulin. As shown in figure 4, administration of diazoxide under the above conditions fails to produce hyperglycemia and there is no significant change in glucose production or uptake.

DISCUSSION

The two main mechanisms proposed for the hyperglycemic effect of diazoxide are a decrease in insulin secretion and an increase in epinephrine secretion. The ability of diazoxide to decrease insulin secretion has been well documented.[12-17] The rapid development of hyperglycemia associated with an acute deficiency of insulin as shown here is due mainly to an increase in hepatic glucose output. This presumably reflects a lessening of the usual restraining effect that insulin exerts on hepatic glucose output.[18] It is also conceivable that increased epinephrine secretion

evoked by diazoxide may stimulate glycogenolysis and thereby increase hepatic glucose output.

It is clear, however, that insulin deficiency alone does not provide an adequate explanation, since diazoxide-induced hyperglycemia has been observed in subjects without a concomitant decrease in plasma insulin levels[19] and in depancreatized animals.[12,13] The present findings are in agreement with this view since maintenance of normal plasma insulin levels by infusion of insulin did not prevent the hyperglycemia induced by diazoxide.

An increase in epinephrine secretion in response to diazoxide has been demonstrated or postulated.[12-15,20] Glycogenolysis induced by endogenous epinephrine would be expected to increase hepatic glucose output and thus increase plasma glucose concentration. However, other studies indicate that increased epinephrine secretion is not essential for the hyperglycemic effect of diazoxide.[13,21] In the present study, infusion of phentolamine in doses sufficient to block hyperglycemia induced by epinephrine (0.2 μg./kg./min.), did not prevent the diazoxide-induced hyperglycemia. It is thus unlikely that the hyperglycemia can be attributed solely to epinephrine.

A strong argument for a role of epinephrine as a mediator of the diazoxide-induced hyperglycemia has been made by Loubatières[13] based on the finding that diazoxide failed to produce hyperglycemia in the depancreatized-adrenalectomized dog. However, glucocorticoid-deficient animals show a characteristic insensitivity to the hyperglycemic effect of injected epinephrine,[22] and, hence, the lack of response to diazoxide is not surprising.

The role of glucagon as a potential factor in the hyperglycemic effect of diazoxide has been explored in this study. The present data reveal that plasma glucagon levels are actually decreased during the period of hyperglycemia, and thus it is unlikely that glucagon contributes to the observed hyperglycemia. Suppression of glucagon secretion by diazoxide has also been reported by Samols and associates,[23,24] who used a perfused isolated canine pancreas preparation.

The preceding discussion dealt with increased hepatic glucose output as a factor in the diazoxide-induced hyperglycemia. The present data also reveal that diazoxide inhibits glucose uptake by tissues. This may be due in part to suppression of insulin secretion, either by a direct action on the beta cells or by stimulation of sympathetic secretion. Nevertheless, replacement of insulin by infusion or blockade of sympathetic activity by phentolamine failed to alter the relative inhibition of glucose uptake produced by diazoxide. It is tempting to postulate that diazoxide may have a direct inhibitory effect on glucose uptake by peripheral tissues. This possibility is supported by the finding that diazoxide inhibits glucose uptake in vitro.[25]

The effect of somatostatin to prevent the diazoxide-induced hyperglycemia was unexpected, particularly since glucagon did not appear to contribute to the hyperglycemia. It is unlikely that this suppression is due to a direct inhibitory effect of somatostatin on hepatic glucose output, since it was found previously[26] that the liver remains sensitive to injected hyperglycemic agents during periods of somatostatin infusion. It is unknown at this time how somatostatin may interfere with the action of diazoxide on the liver. Possible sites of interaction may include opposing effects on calcium flux or on nucleotide formation, but this remains to be explored.

ACKNOWLEDGMENTS

Investigations were supported by Public Health Service Research Grant AM 10188 from the National Institute of Arthritis, Metabolic, and Digestive Diseases.

REFERENCES

[1]Diazoxide and the treatment of hypoglycemia. Ann. N.Y. Acad. Sci. *150*:Art. 2, 191-467, 1968, Smith, H. M., and Weyer, E. M., Eds.

[2]deBodo, R. C., Kurtz, M., Ancowitz, A., and Kiang, J. S.: Anti-insulin and diabetogenic actions of purified anterior pituitary growth hormone. Am. J. Physiol. *163*:310-18, 1950.

[3]Altszuler, N., Barkai, A., Bjernkes, C., and Gottlieb, B.: Glucose turnover values in the dog obtained with various species of labelled glucose. Am. J. Physiol. *229*:1662-67, 1975.

[4]Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69-73, 1974.

[5]Washko, M. E., and Rice, E. W.: Determination of glucose by an improved enzymatic procedure. Clin. Chem. 7:542-45, 1961.

[6]Hales, C. N., and Randle, P. J.: Radioimmunoassay of insulin with antibody precipitate. Biochem. J. *88*:137-46, 1963.

[7]Faloona, G. R., and Unger, R. M.: Glucagon. *In* Methods of Hormone Immunoassay. Jaffe, B. M., and Berman, H. R., Eds., New York, Academic Press, 1974, pp. 317-30.

[8]Steele, R., Wall, J., deBodo, R. C., and Altszuler, N.: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am. J. Physiol. *187*:15-24, 1956.

[9]Steele, R.: Influence of glucose loading and of injected insulin on hepatic glucose output. Ann. N.Y. Acad. Sci. *82*:420-30, 1959.

[10]Cowan, J. S., and Hetenyi, G., Jr.: Glucoregulatory re-

sponses in normal and diabetic dogs recorded by a new tracer method. Metabolism *20*:360-72, 1971.

[11]Steele, R., Rostami, H., and Altszuler, N.: A two-compartment calculator for the dog glucose pool in the nonsteady state. Fed. Proc. *33*:1869-79, 1974.

[12]Tabachnick, I. I. A., Gulbenkian, A., and Seidman, F.: The effect of a benzothiadiazine, diazoxide, on carbohydrate metabolism. Diabetes *13*:408-18, 1964.

[13]Loubatières, A., Mariani, M. M., and Alric, R.: The action of diazoxide on insulin secretion, medullo-adrenal secretion, and the liberation of catecholamines. Ann. N.Y. Acad. Sci. *150*:226-41, 1968.

[14]Kvam, D. C., and Stanton, H. C.: Studies on diazoxide hyperglycemia. Diabetes *13*:639-44, 1964.

[15]Graber, A. L., Porte, D., Jr., and Williams, R. H.: Clinical use of diazoxide and mechanism for its hyperglycemic effect. Diabetes *15*:143-48, 1966.

[16]Seltzer, H. S., and Crout, J. R.: Obliteration of insulin secretion by diazoxide and its reversal by tolbutamide. Diabetes *15*:523, 1966.

[17]Fajans, S. S., Floyd, J. C., Jr., Thiffault, C. A., Knopf, R. F., Harrison, J. S., and Conn, J. W.: Further studies on diazoxide suppression of insulin release from abnormal and normal islet tissue in man. Ann. N.Y. Acad. Sci. *150*:261-80, 1968.

[18]Altszuler, N., Steele, R., Tobin, J., Rathgeb, I., and deBodo, R. C.: Effect of anti-insulin serum on glucose production and uptake in dogs. Excerpta Medica, International Congress Series *74*:168, 1954.

[19]Bleicher, S. J., Chowdhurry, F., Podolsky, S., Fleishman, P., and Goldner, M. G.: Studies on diazoxide-induced hyperglycemia: an extrapancreatic mechanism. Ann. N.Y. Acad. Sci. *150*:294-302, 1968.

[20]Staquet, M., Yabo, R., Viktora, J., and Wolff, F.: An adrenergic mechanism for hyperglycemia induced by diazoxide. Metabolism *14*:1000-09, 1965.

[21]Seltzer, H. S., and Crout, J. R.: Effects of different benzothiadiazines and catecholamines on insulin secretion. Program, 48th Meeting Endocrine Society, Chicago, Ill., June 20-22, p. 78, 1966.

[22]deBodo, R. C., and Sinkoff, M. W.: Anterior pituitary and adrenal hormones in the regulation of carbohydrate metabolism. Rec. Prog. Horm. Res. *8*:511-70, 1953.

[23]Samols, E., and Harrison, J.: Suppression of glucagon secretion by diazoxide. Clin. Res. *23*:330A, 1975.

[24]Samols, E., Harrison, J., and Arimura, A.: Diazoxide suppresses glucagon secretion in vitro. Clin. Res. *24*:424A, 1976.

[25]Barnett, C. A., and Whitney, J. E.: The effect of diazoxide and chlorothiazide on glucose uptake in vitro. Metabolism *15*:88-93, 1966.

[26]Altszuler, N., Gottlieb, B., and Hampshire, J.: Interaction of somatostatin, glucagon, and insulin on hepatic glucose output in the normal dog. Diabetes *25*:116-21, 1976.