Glutamate-Induced Hyperglycemia.* (31899)

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During the course of other investigations glutamic acid was noted to produce significant hyperglycemia in normal rats. Further studies have been conducted in an effort to define the mechanism of this unexpected response, and the results are presented here.

Materials and methods. Male Sprague-Dawley rats, weighing between 200 and 300 g, were used in all experiments, and assigned to groups with the aid of a table of random numbers. Experiments were generally performed after an overnight fast (14 hours), but some studies were carried out following 48 hours of fasting. Test substances were prepared as 0.15 molar aqueous solutions, and injected intraperitoneally. Blood was obtained from non-anesthetized animals by cardiac puncture either 30 or 60 minutes after administration of the test substances. Glucose concentration was determined by the glucose-oxidase method (Glucostat, Worthington Biochemical Corp.).

Adrenalectomized animals were maintained on standard laboratory rat chow, but their drinking water was replaced with 0.9% sodium chloride. They were used on the fourth post-operative day. Spontaneous ACTH activity was suppressed by administration of dexamethasone, 25 μg per 100 g body weight, 2 hours prior to injection of test substances. This dose has been found to inhibit the corticotrophin-releasing response to non-specific stress(1).

Pyruvate-2-C^{14}, alanine-U-C^{14}, and glutamate-3,4-C^{14}, obtained from New England Nuclear Corp., were added to non-labeled solutions of pyruvate, alanine and glutamate. The test solutions were injected intraperitoneally into rats fasted 14 hours. Each rat received 0.4 moles of "cold" and 2 μc of tracer amounts of C^{14}-labeled substrate/100 g body weight. Blood was obtained 30 minutes after injection. Plasma was separated into 2 aliquots: one used to measure plasma glucose concentration (glucose oxidase) and the other to isolate glucose by thin layer chromatography(2). The glucose spot was scraped off the plate, and eluted; the eluate was divided into 2 aliquots: one aliquot was used for determination of radioactivity (Packard Liquid Scintillation Counter), and the other for glucose measurement (glucose oxidase).

Results and discussion. The degree of hyperglycemia produced by glutamate is seen in Table I. Elevation of plasma glucose concentration induced by glutamate was significantly greater (t-test) than that following equimolar amounts of alanine at 30 and 60 minutes or glucose 60 minutes after injection (P in all cases was <0.01).

Effects of increasing amounts of glutamate on plasma glucose concentration at 60 minutes are seen in Fig. 1. Although 0.05 mmoles/100 g body weight produced a small elevation of plasma glucose, significant hyperglycemia was not achieved until the dose reached 0.1 mmoles/100 g body weight. Maximum hyperglycemia was reached with 0.2 mmoles/100 g body weight, and doubling this dose did not lead to any significant further elevation. Detailed analysis of the glutamate dose response curve made it seem unlikely that the rise in plasma glucose concentration was solely due to conversion of glutamate to glucose. Plasma glucose concentration increased from 88 to 173 mg%, an increment of 85 mg%, when the dose of glutamate was raised from 0.01 to 0.20 mmoles/100 g body weight. The additional amount of glutamate responsible for this rise in plasma glucose would be equal to 0.456 mmoles in a 240 g rat (0.19 mmoles/100 g body weight $\times$ 2.4). Since 1 mole of glutamate is converted to 1 mole of phosphoenol-pyruvate, and 2 moles of the latter are required for 1 mole of glucose, the additional glutamate could produce, at most, 0.228 mmoles of glucose. If glutamate was entirely converted to glucose, and if none of this newly formed glucose was utilized, the net increment in total body glucose would

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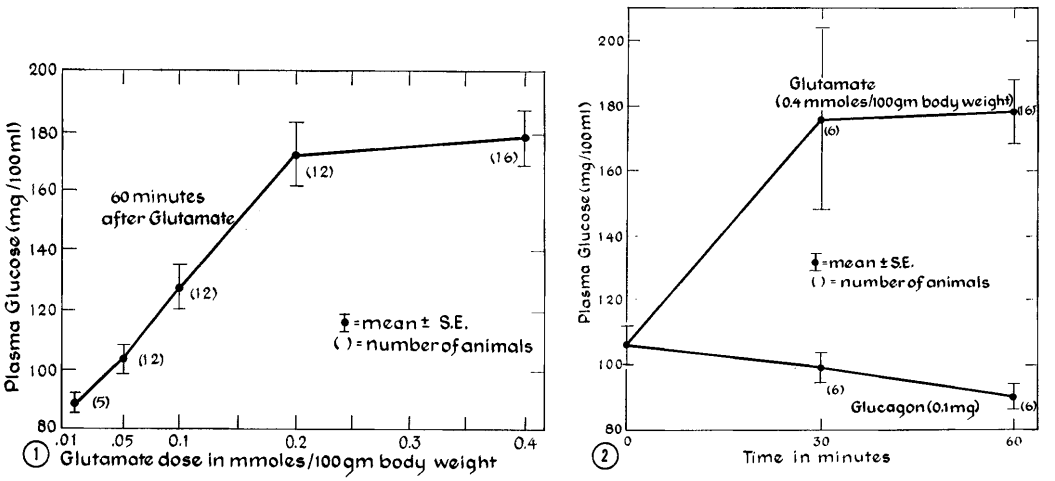

Fig. 1. Response of plasma glucose concentration to administration of increasing amounts of glutamate.

Fig. 2. Comparison of glucagon and glutamate adminstration on plasma glucose concentration of rats fasted for 14 hr.

equal 41 mg (0.228 mmoles × 180 mg/mmole). As stated above, the effect of the additional 0.19 mmoles of glutamate was to increase plasma glucose concentration 85 mg%. If one assumes that glucose is confined to the extracellular space, and that its volume of distribution approximates 25% of body weight, *i.e.*, 60 ml in a 240 g rat, the net increase in total body glucose produced by the additional 0.19 mmoles per 100 g body weight would be 51 mg (0.85 mg/ml × 60 ml), or 10 mg more than could be accounted for by the quantitative conversion of glutamate to glucose and complete inhibition of glucose removal. Consequently, the possibility that glutamate produced hyperglycemia solely by its conversion to glucose seemed unlikely.

The possibility that glutamate produces hyperglycemia by conversion to glucose was further studied by measuring the relative incorporation of several C^{14}-labeled substrates into glucose. Rats fasted 14 hours received i.p. injections of either alanine, pyruvate or glutamate solutions, to which C^{14}-labeled tracer amounts of the same substrate had been added. The percent of administered isotope present as glucose 30 minutes later was determined, and results are seen in Table II. The amount of radioactivity in glucose in animals receiving C^{14} glutamate was not greater than that following administration of C^{14} alanine or pyruvate.

TABLE I. Response of Plasma Glucose Concentration to Intraperitoneal Administration of Glucose, Glutamate and Alanine (.4 mmole/100 g Body Weight). Fasting plasma glucose concentration = 102 ± 3.2 (8).

	Plasma glucose (mg % ± SE)	
Substrate	30 minutes	60 minutes
Glucose	164 ± 7.2 (9)	133 ± 4.1 (16)
L-glutamate	176 ±28.0 (6)	178 ± 9.2 (16)
L-alanine	121 ± 7.8 (6)	130 ± 4.2 (9)

( ) = No. of animals.

These results suggested that glutamate-induced hyperglycemia most likely resulted from glycogenolysis or gluconeogenesis. The possibility that glutamate produced hyperglycemia by stimulating glycogenolysis was investigated next by comparing the effects of glutamate with those of a large dose of glucagon (0.1 mg) in rats fasted 14 hours. The results are seen in Fig. 2. A maximal dose of glucagon did not increase plasma glucose in these rats, suggesting that glycogen stores are severely depleted in a 14-hour fasted rat. Since the effect of glutamate was still evident, it seems unlikely that glutamate acts by increasing conversion of glycogen to glucose.

The possibility that glutamate-induced hyperglycemia results from stimulation of the pituitary-adrenal axis was then examined. Results are seen in Table III, and indicate that adrenalectomy abolished the hyperglycemic

TABLE II. Incorporation of Labeled Precursors into Glucose.

Isotope injected	Non-labeled compound injected (.4 mmole/100 g)	No. of animals	% Isotope in glucose 30 min after injection (mean ± SE)*
Alanine-U-C^{14}	Alanine	4	8.7 ± .54
Pyruvate-2-C^{14}	Pyruvate	4	6.8 ± 1.07
Glutamate-3,4-C^{14}	Glutamate	4	6.1 ± 1.02

* Calculated on basis of plasma glucose concentration, plasma glucose specific activity, and assumption that glucose space is 25% of body wt.

TABLE III. Effect of Adrenalectomy and ACTH Suppression on Plasma Glucose Response to Glutamate (.4 mmole/100 g Body Weight) in Rats Fasted 14 Hours.

Experimental condition	No. of animals	Elevation of plasma glucose (mg %),* mean ± SE
Control	12	64 ± 9
Adrenalectomy	6	6 ± 4
Dexamethasone (25 μg/100 g body wt)	6	58 ± 11

* Rise in plasma glucose 60 min after receiving glutamate.

by increasing new glucose production, and suggest that this may result from stimulation of gluconeogenesis. The possibility that this action may be related to the recent suggestion (3) that gluconeogenesis is dependent upon transport of 4-carbon fragments across the mitochondrial membrane is currently being investigated.

Summary. Plasma glucose rose linearly with increasing amounts of glutamate up to a maximal dose of 0.2 mmoles/100 g body weight. The glutamate effect could not be explained by stoichiometric conversion of glutamate to glucose. Glutamate produced hyperglycemia after 14-hour fast, at which time glucagon had no effect on glucose concentration. Adrenalectomy abolished the glutamate effect; ACTH suppression with dexamethasone did not. Glutamate supplementation increased the plasma glucose response to pyruvate in excess of the additive effects of either substrate alone. It is suggested that glutamate produces hyperglycemia by stimulating new glucose production, and that this most likely results from increased gluconeogenesis.

TABLE IV. Effect of Glutamate Supplementation on Plasma Glucose Response to Pyruvate Administration.

Group	No. of animals	Elevation of plasma glucose (mg %),* mean ± SE
Pyruvate (.2 mmole/100 g body wt)	14	9 ± 3
Glutamate (.2 mmole/100 g body wt)	21	32 ± 6
Pyruvate (.2 mmole) + glutamate (.2 mmole/100 g body wt)	28	72 ± 8

* Rise in plasma glucose 60 min after injection.

effect of glutamate. Suppression of ACTH activity did not alter the glutamate effect. These results indicate that although intact adrenals are necessary for glutamate to produce hyperglycemia, acute stimulation of the pituitary-adrenal axis is not the cause of this phenomenon.

Finally, an attempt was made to demonstrate directly that glutamate stimulates gluconeogenesis. These results are seen in Table IV. Pyruvate administration to rats fasted 48 hours produced a slight increase in plasma glucose concentration. The concomitant administration of pyruvate and glutamate markedly increased plasma glucose concentration, and this rise was greater than could be accounted for by the cumulative effects of pyruvate plus glutamate (t-test, $P<.02$). These results further support the thesis that glutamate produces hyperglycemia

1. Kendall, J. W., Jr., Endocrinology, 1962, v71, 452.

2. Nikkilä, E. A., Ojala, K., Life Sci., 1964, v3, 243.

3. Lardy, H. A., Paetkau, V., Walter, P., Proc. Nat. Acad. Sci., 1965, v53, 1410.

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