Profile of Insulin Release Due to Intrapancreatic Glyburide Infusion

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SUMMARY

Intrapancreatic glyburide infusion in dogs (0.02 mg. per kg. of body weight) for five minutes provoked immediate, marked insulin release into the portal venous blood. Compared to control animals, the normal intrinsic insulin levels were exaggerated at intervals of approximately eighty minutes. Lack of immediate hypoglycemia suggests that the insulin release is merely secondary in contributing to the antidiabetic property of this agent. An amount of tolbutamide fifty times as great caused a weak, delayed insulin response. The delayed blood glucose response is probably caused by the combined action of glyburide and circulating insulin. DIABETES *21*:209-15, April, 1972.

The major antidiabetic property of the sulfonylureas, to produce hypoglycemia, is thought to be caused by stimulation of insulin secretion. Intravenous infusion of tolbutamide enhanced peripheral plasma venous insulin levels and reduced blood glucose in both humans[1] and dogs.[2,3] The newest and most potent derivative, glybenzcyclamide, likewise provoked pancreatic venous insulin output and hypoglycemia within one-half hour.[4] These studies failed to prove that this agent directly stimulated the pancreatic islets, since some intermediary action might have been involved, and did not correlate the kinetics of insulin secretion with existing glucose concentration. Therefore, our study was designed to localize the magnitude of the direct stimulus of this new drug to the pancreas, and to compare the chronological sequence of insulinemic and glycemic responses with the effect of its parent compound, tolbutamide.

METHODS

Mongrel dogs weighing 15 to 22 kg. were anesthetized intravenously with sodium Nembutal. For pancreatic infusion, a polyethylene catheter was inserted into the right gastroepiploic artery, so that the pancreaticoduodenal artery was perfused by the technic described previously.[5-7] A second tube was threaded via a mesenteric vein to a point in the portal vein beyond the entrance of the pancreaticoduodenal vein. This allowed us to determine the effect of a five-minute infusion of the experimental agent on insulin efflux from the pancreas. The glybenzcyclamide* was dissolved with a small amount of 1 N NaOH in alcohol and water (1 part in 100) and made up with saline to a concentration of 0.1 mg. per ml. Dose was 0.02 mg. per kg. of body weight, and rate of infusion was 1 ml. per min. Pancreas-injected controls received the same vehicle but the glybenzcyclamide was omitted. Other dogs received tolbutamide, 1 mg. per kg. of body weight, in a concentration of 5 mg. per ml. of saline for a mean dose of 20 mg. Blood samples were withdrawn from the femoral artery and portal vein fifteen and two minutes before, and at intervals of five to thirty minutes after infusion for four hours. Frozen plasma was stored at —10° to —20° C. prior to analysis. Immunoreactive insulin[8] and glucose[9] were determined in duplicate.

RESULTS

Preinfusion glucose and insulin values in both venous and arterial blood were similar in the two groups, as well as mean dog weights, so that comparison of experimental and control data following infusion is justified (table 1). Glyburide produced immediate insulin release in all six experimental animals (figure 1). Mean portal venous blood insulin was significantly enhanced compared to that in control dogs five minutes after beginning the infusion, while the concomitant glucose concentrations were similar. Comparison with controls of the integrated insulin responses in both femoral arterial and portal venous blood for the four-hour period

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*1 (p-(2-(5-chloro-o-anisamido) ethyl) phenylsulfonyl)-3-cyclohexylurea, or U-26452, Lot No. 8707-KGW-2D, kindly supplied by Dr. William Dulin, The Upjohn Co., Kalamazoo, Mich.

TABLE 1

Preinfusion insulin and glucose in portal venous (P.V.) and femoral arterial (F.A.) blood in intrapancreatic infusions*

Agent	No. of dogs	Site of determination	Insulin (μU./ml.) −15′	Insulin (μU./ml.) −2′	Glucose (mg./100 ml.) −15′	Glucose (mg./100 ml.) −2′
Glyburide	6	P.V.	60 ± 10	78 ± 16	111 ± 6	109 ± 6
		F.A.	40 ± 6	40 ± 8	116 ± 5	120 ± 10
Control	4	P.V.	66 ± 22	56 ± 16	123 ± 8	127 ± 3
		F.A.	40 ± 11	28 ± 3	129 ± 4	136 ± 9

*The differences between respective experimental and control means ± Standard Error of Means ($\bar{x} \pm$ S.E.M.) are not significant by Student "T" test.

in the two groups revealed a significant ninefold and threefold respective ($p<0.05$) response due to the experimental agent.

There is a marked correlation in the profiles of glucose in both venous and arterial blood at the same time periods in control and experimental groups, although there was no significant difference between them until the ninety and 120-min. samplings. The mean integrated ratios for insulin and glucose (I/G) calculated for the glyburide and control dogs in portal venous blood for the entire four-hour period were 1.88 ± 0.17 and 0.55 ± 0.07 respectively. In most cases this significant increase above control levels was due to the exaggerated insulin secretion, as noted in the portal venous insulin peaks, which are significant at 5, 120, 180 and 240 min. Although the integrated insulin response was also significant in the arterial blood and similar in pattern to the venous blood, the concentration failed to fully reflect the marked enhancement of islet secretory activity (table 2). Peripheral arterial insulin was significantly enhanced in experimental vs. control dogs at 10, 20, 90, 120, 150, 210 and 240 min., whereas these levels were only reflected in arterial glucose at ninety and 120 min. (figure 2). These lower levels did correspond to the maximal insulin levels of the secondary phase of secretion, which was actually greater in magnitude than the primary phase.

The peripherally infused animals also exhibited an immediate portal venous insulin response to glyburide, maximal ten to twenty minutes after the beginning of infusion (figure 3). Although this was reflected in the femoral arterial blood, a second peak after ninety to 120

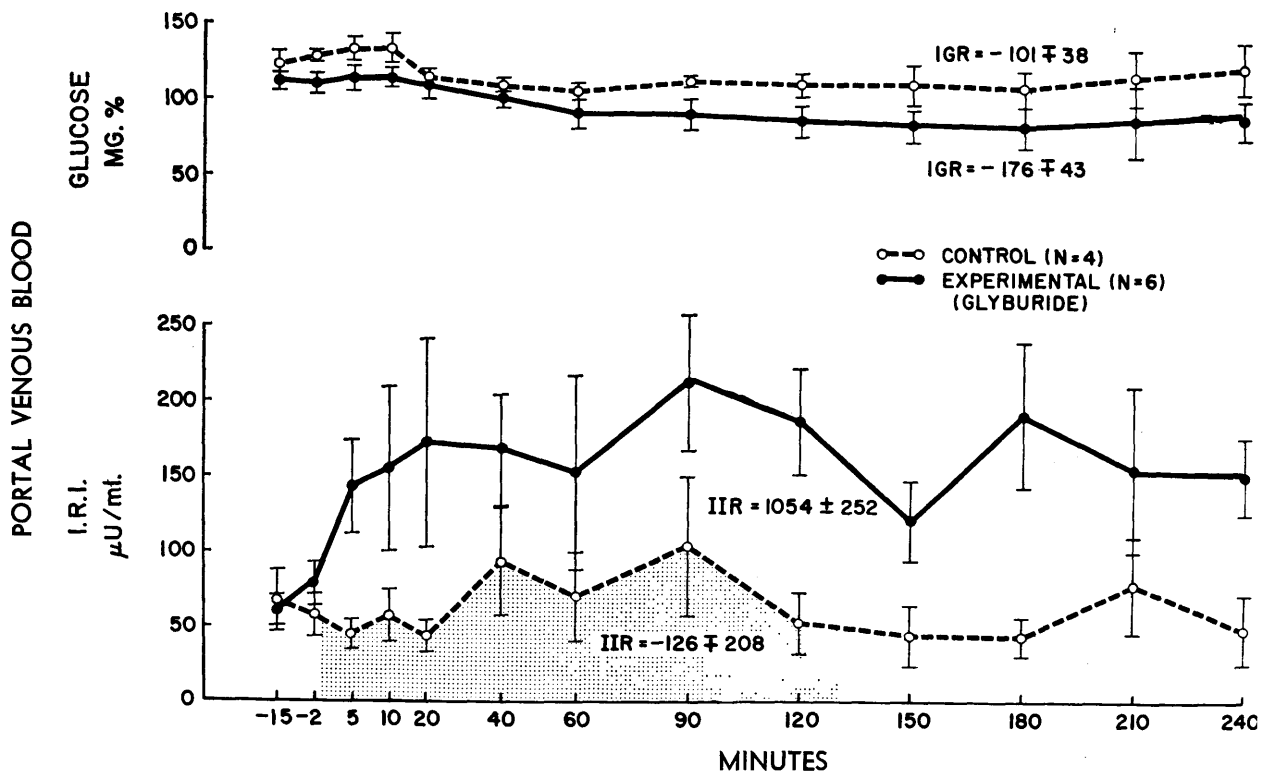

FIG. 1. Portal venous blood insulin and glucose responses to intrapancreatic infusion of glyburide compared to responses in control animals infused with the vehicle alone. IIR refers to integrated insulin and IGR to integrated glucose response, and vertical bars refer to S.E.M.

TABLE 2

Integrated insulin (I) and glucose (G) responses in portal venous (P.V.) and femoral arterial (F.A.) blood to intrapancreatic infusion of glyburide or vehicle*

Determination	Site	Glyburide (6)	Control (4)	p value †
I	P.V.	1,054 ± 252	−126 ± 208	<0.01
G	P.V.	−176 ± 43	−101 ± 38	n.s.
I	F.A.	169 ± 40	−96 ± 66	<0.01
G	F.A.	−168 ± 45	−136 ± 72	n.s.

* The values are the Mean ± S.E.M. for the integrated insulin (μU./ml.) and glucose (mg./100 ml.) responses.
† Student "T" test.

min. was not. The blood glucose dropped gradually, mainly in conjunction with the secondary phase of insulin release, but not to hypoglycemic levels. The profile of insulin release was again similar to that of the pancreatic-infused animals, but the magnitude was only half as great.

By summing the insulin data for the first hour-period after infusion and plotting the increments to fit a log normal distribution, one may determine the time at which the initial ($\bar{\bar{t}}_0$) response is detectable in the experimental dogs. In portal venous blood, $\bar{\bar{t}}_{01}$ is twelve seconds from the start of infusion, and the mean time ($\bar{\bar{t}}_1$) is 15.2 min. Applying the same analysis to the secondary peak (sixty to eighty minutes) without correction for existing insulin and its half-life indicates that $\bar{\bar{t}}_{02}$ is at 8.5 min. Since $\bar{\bar{t}}_2$ is 96.5 min., the insulin peaks seem to appear at approximately eighty-minute intervals from the first peak.

Injection of tolbutamide into the pancreas produced a weak enhancement of pancreatic insulin output, delayed until after ninety minutes (figure 4). Except for the two-hour value, differences from control venous insulin levels were not significant at the individual time intervals, but the integrated insulin response was definitely greater than the control level. This was mainly due to the late insulinemia occurring after ninety minutes. The mean I/G calculated for the entire four-hour period for the tolbutamide infusion was 0.78 ± 0.05 ($\bar{X}$ ± S.E.M.), which is not significantly different from the control (0.55±0.07). The quantity of output was only half of that observed from the glyburide. As with the newer agent, the intrinsic pattern of insulin release from tolbutamide bore a similarity to the control profile, but this was true only after ninety minutes. The late blood glucose fall in portal venous blood seemed to correlate

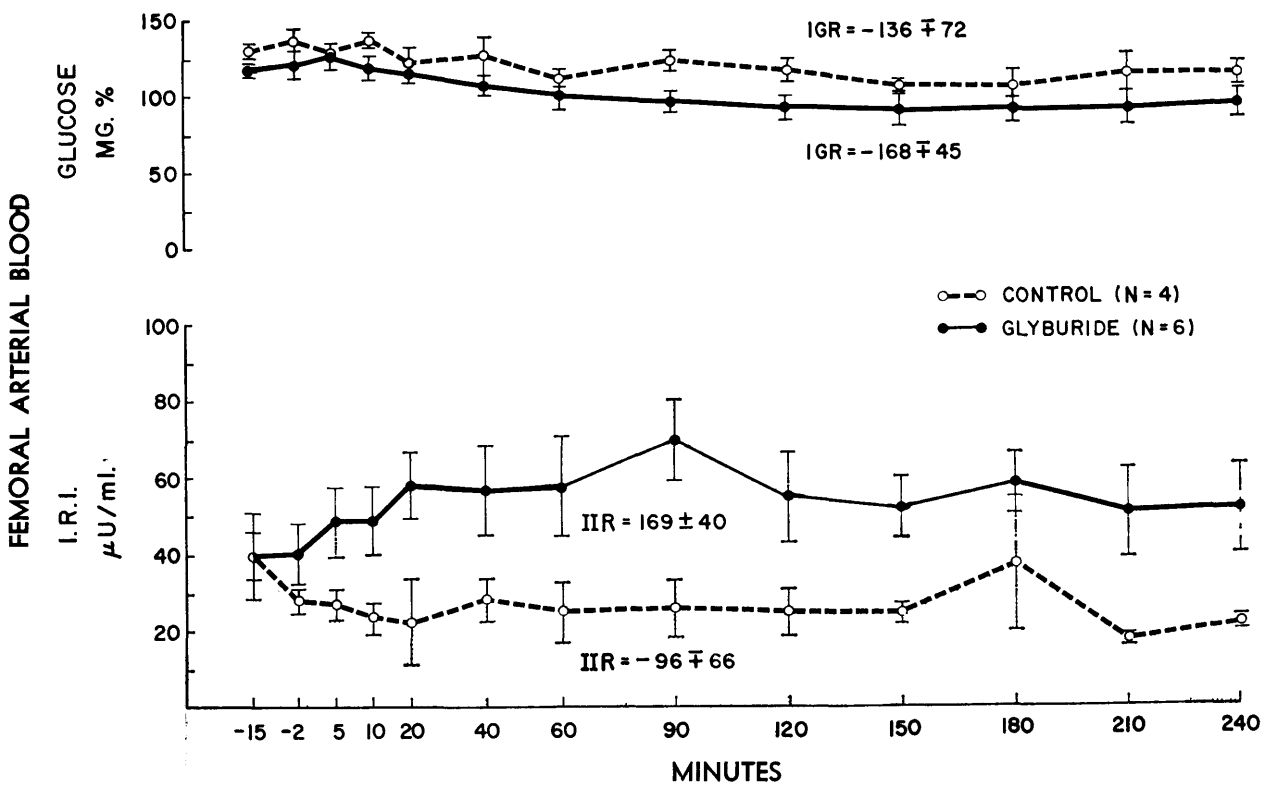

FIG. 2. Femoral arterial blood insulin and glucose responses to intrapancreatic infusion of glyburide or control vehicle.

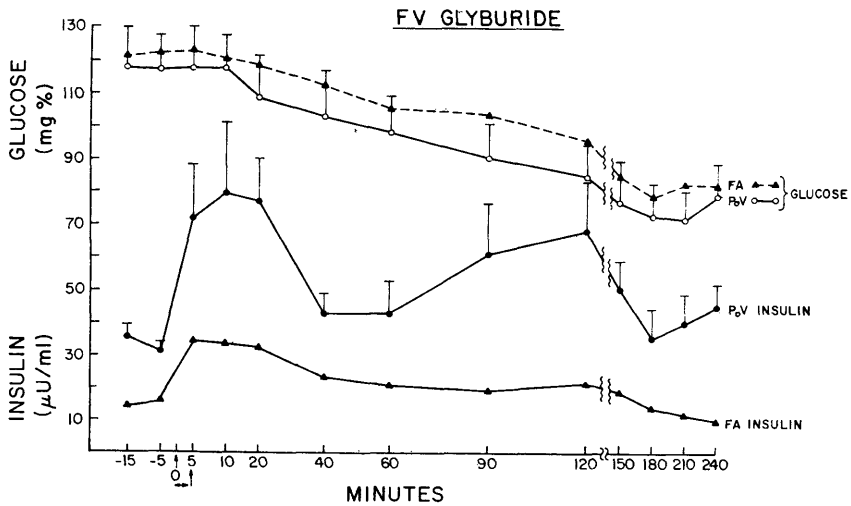

FIGURE 3

Portal venous and femoral arterial blood insulin and glucose concentrations following glyburide infusion of the femoral vein.

with the insulinemia, even though the differences were not significant.

Although the excursions of the portal venous insulin levels are more profound in the glyburide-injected group, their chronological similarity to those of the other three groups is striking (figure 5). The primary cycle of insulin release was delayed in the control and absent in the tolbutamide animals. The secondary phase appeared in all groups, and the tertiary phase at 80 to 120 min. in all except the peripherally infused animals. These cyclic responses are not statistically verifiable when the sampling is from the femoral artery (figure 6) and only with the glyburide do we see any periodicity resembling the portal vein profile. Blood glucose responses,

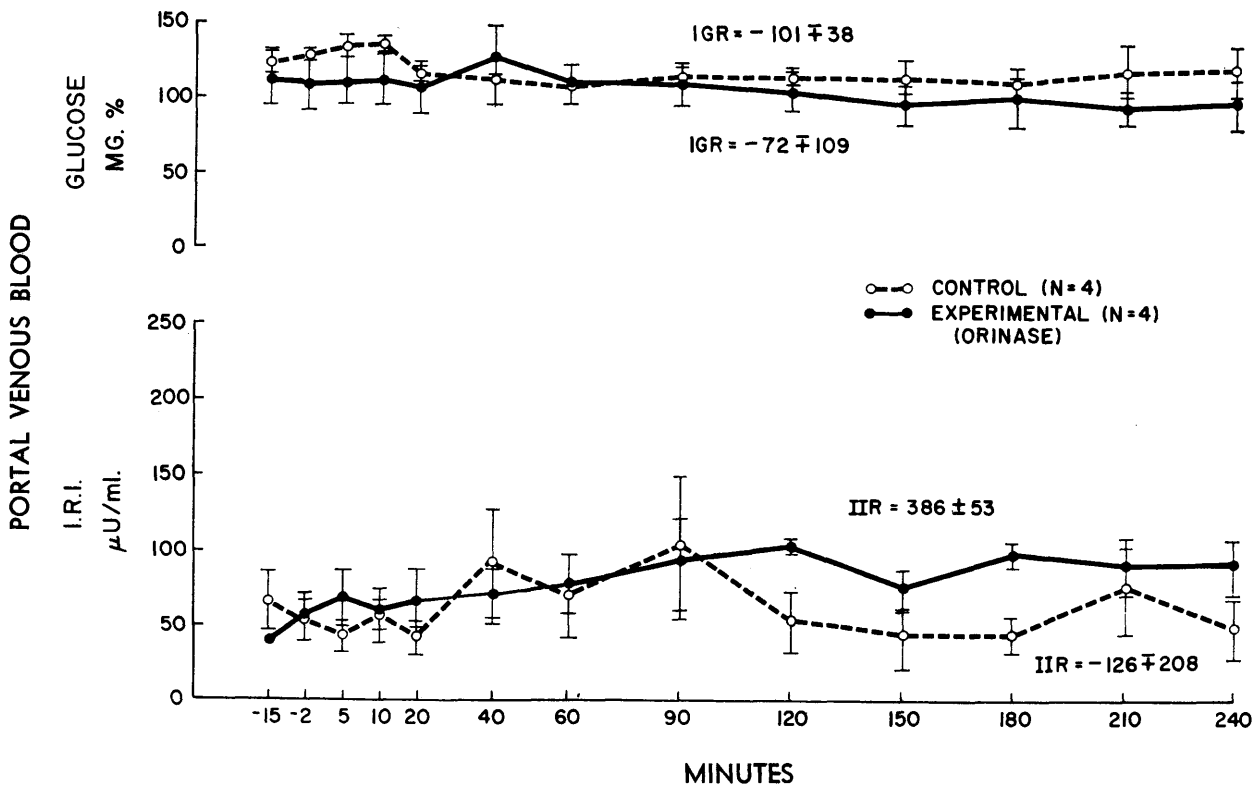

FIG. 4. Portal venous blood insulin and glucose levels following intrapancreatic infusion of tolbutamide compared to responses in control animals infused with the vehicle alone.

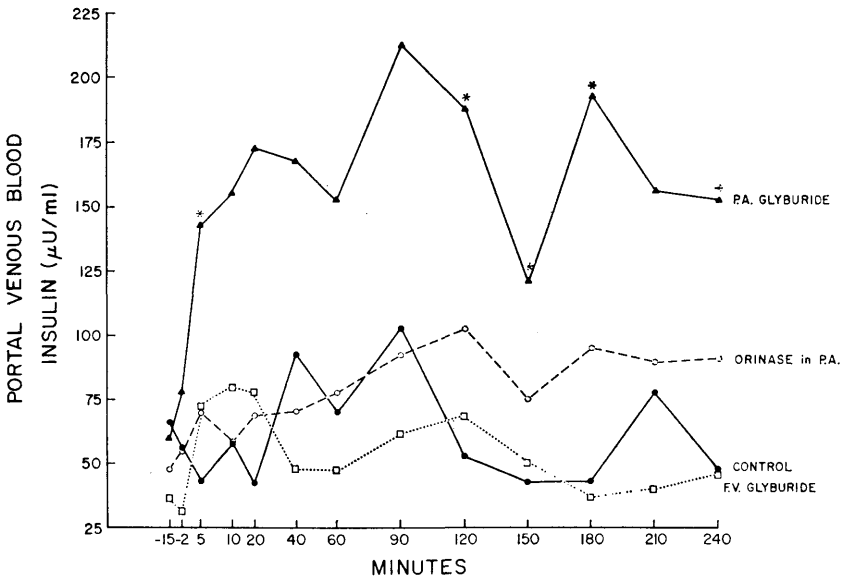

FIGURE 5

Comparison of portal venous blood insulin concentrations in dogs injected intrapancreatically or peripherally with glyburide, tolbutamide or control vehicle.

portal venous and femoral arterial, are definite only following glyburide infusion into the pancreatic artery or femoral vein (figures 7 and 8). The differences become prominent after one hour and are more pronounced with the peripheral infusions.

DISCUSSION

Intrapancreatic glyburide consistently caused cyclic insulin levels having maximum increments roughly every eighty minutes after the initial phase. The early pulse may have represented release of stored insulin[10,11] and was not due to hyperglycemia. It appeared as soon as twelve seconds from the start of the infusion with a mean efflux time of fifteen minutes. The second pulse was calculated to start 8.5 min. later and correlated directly and chronologically with endogenous variations in control animals injected with the vehicle only. This indicates that glyburide enhanced the normal intrinsic beta cell function. The late surges may reflect normal secretory mechanisms involved in release of newly synthesized insulin, occurring long after the pancreatic stimulus was withdrawn.

When one looks at the ratios of insulin to glucose there is a significant increase in the glyburide compared to the control group, and the insulin levels correlate with glucose at all intervals with the exception of the five- and ten-minute samples. The failure of the initial insulin release to correlate negatively with the glucose levels could be interpreted either as a poor response of the target tissue or as lack of effectiveness of stored insulin. The findings are supported by studies showing that physiologic levels of insulin infused into dogs failed to reduce the blood glucose.[18] The hypoglycemia becomes significant only at ninety and 120 min. when this agent is administered directly into the pancreas. The same amount of glyburide when infused peripherally provoked an immediate but insignificant amount of insulin release resulting in an even lower glucose value

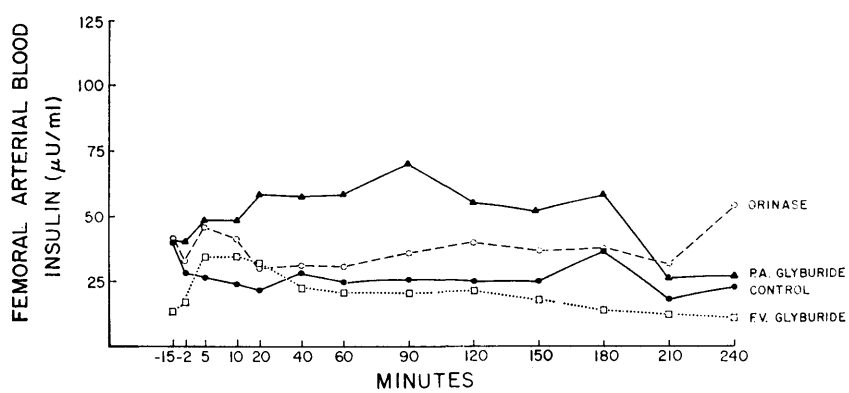

FIGURE 6

Femoral arterial blood insulin responses in animals infused with the various agents at the sites indicated.

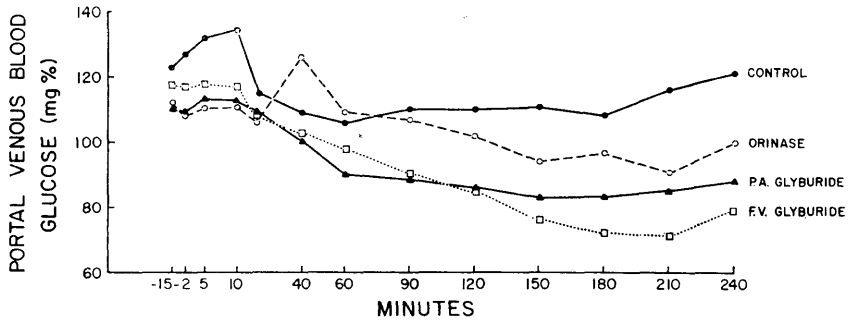

FIGURE 7

Portal venous blood glucose responses in animals infused with the various agents as indicated.

at two hours. Therefore, glyburide has two actions. The first is related to insulin release; the second, to glucose production or consumption. Thus, the lack of an immediate blood glucose response to the early insulin release indicates that pancreatic islet function alone is not sufficient for control of glycemia.

It has been suggested that the delayed hypoglycemia from tolbutamide is thought to be caused by recirculation of biologically active insulin through the liver.[16] Hypoglycemia occurring one to three-and-one-half hours after combined tolbutamide and insulin infusion into the portal vein may be explained by the agents' potentiation of the subsequent endogenous insulin in the liver.[17] Similarly, glyburide-induced hypoglycemia may

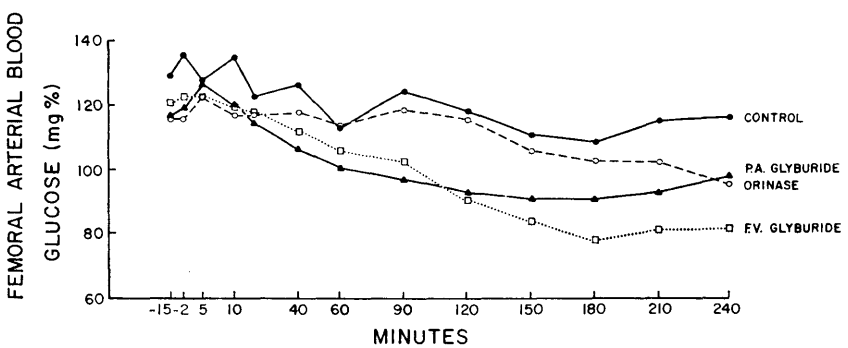

FIGURE 8

Femoral arterial blood glucose responses in animals infused with the various agents as indicated.

not appear until the concentrations of insulin and circulating glyburide have combined to reduce hepatic glucose output. Further studies must be performed on the dynamics of glyburide disposition, with reference to preferential absorption from the circulation by certain tissues and the resulting effect on glycemia.

In order to estimate the potency of an increased concentration of intrapancreatic glyburide, it may be of interest to compare the magnitudes of blood glucose responses from data available in the literature. The minimal effective dose of intravenous glyburide, 0.025 mg. per kg. of body weight, similar to the one employed in our experimental dogs, produced a slight (15 per cent) fall in blood glucose in one hour which is comparable to the 20 per cent shown in the data.[18] An amount ten times that much caused up to sixfold increase in peripheral venous plasma insulin in dogs with a 35 per cent reduction in blood glucose, both maximal at thirty to sixty minutes after injection.[4] An amount twenty times that much enhanced pancreatic insulin release into the portal vein fivefold, maximal at the end of two hours.[18] The delayed responses were attributable to the route of delivery being intravenous rather than intrapancreatic. Our data indicate that the potency of the direct pancreatic stimulus was at least doubled for the four-hour period compared to the intravenous infusion. Since pancreatic venous blood is diluted two to three times by mesenteric blood circulating through the portal vein, our venous blood measurements did not reflect the maximal concentration actually reached. The opinion is expressed that pancreatic venous insulin measurements are superior to the peripheral estimations commonly employed,[12] but unless a bypass is provided one must sacrifice normal physiologic control exerted by the endogenous insulin. In our experiments a ninefold integrated rise in portal venous insulin was damped to a threefold increase as viewed from the results on femoral arterial samples, and the multiphasic nature of

the response was completely masked in the femoral artery due to dilution and absorption of insulin. Therefore, collection limited to peripheral venous blood in human studies may fail to identify minor fluctuations in islet activity.

Under similar experimental conditions, glucose (1 gm.) injected for thirty minutes produced a biphasic response to portal venous insulin maximal at five and thirty minutes, with a late rise at ninety minutes.[7] Kanazawa et al.,[14] employing intravenous instead of intrapancreatic glucose, also found two or more peaks from twenty to ninety minutes after infusion. Intrapancreatic casein hydrolysate (1 gm.) caused a single large peak maximal at fifteen minutes and comparable in amount to that from glucose, with a secondary rise at sixty minutes.[7] Glucagon (10 μg.) injected into the pancreatic artery for five minutes caused a primary peak which appeared later than with the glyburide (fifteen minutes after infusion) and an enhanced secondary response in ninety minutes.[15] The chronological similarity between the early responses to the different stimulatory agents is noteworthy. As in the case of glyburide, the magnitude of the secondary surge of insulin release due to these agents coming one to two hours after infusion is comparable to or greater than that of the early pulse. This indicates that after the initial release of insulin has been accomplished, the pancreatic islets can release additional insulin within an hour. A similar pattern in fluctuations of insulin secretory activity in control animals lends credence to a mechanism of action common to these various stimuli which involves exaggeration of a normal intrinsic sequence of events.

In conclusion, our findings indicate that glyburide enhances the normal intrinsic islet activity, causing maximal insulin levels at approximately eighty-minute intervals following the immediate initial response. The pronounced portal venous insulin secretory response within five minutes after pancreatic arterial infusion is reflected to some extent in femoral arterial blood, but is not associated with an appropriate blood glucose fall. This suggests that the primary antidiabetic action of this agent, to produce hypoglycemia, is not caused directly by the amount of insulin released. A very small dose (0.02 mg. per kg. of body weight) of glyburide is more rapid and potent in directly stimulating insulin release than an amount of tolbutamide fifty times as great. The profile of insulin secretion from this dose of tolbutamide also correlated with that seen in control animals, although the hypoglycemia was rather weak and delayed until after ninety minutes. The delay in reduction of blood glucose from agents may be due to concomitant release of another component such as glucagon or to an inhibition of the hepatic effects of insulin on glycogenesis.

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REFERENCES

1 Varsano-Aharon, N., Eschemendia, E., Yalow, R. S., and Berson, S. A.: Early insulin responses to glucose and to tolbutamide in maturity-onset diabetes. Metabolism *19*:409-17, 1970.

2 Seltzer, H. S.: Quantitative effects of glucose, sulfonylureas, salicylate and indole-3-acetic acid on the secretion of insulin activity into pancreatic venous blood. J. Clin. Invest. *41*:289-300, 1962.

3 Colwell, A. R., Jr., and Metz, R.: Secretion of an insulin-like substance into the pancreatic vein. A result of tolbutamide administration. Diabetes *11*:504-08, 1962.

4 Sirek, O. V., Vigas, M., Niki, A., Niki, H., and Sirek, A.: Beta-adrenergic stimulation and insulin release in dogs following H B 419. Diabetologia *5*:207-10, 1969.

5 Colwell, A. R., Jr., and Colwell, J. A.: Pancreatic action of the sulfonylureas. J. Lab. Clin. Med. *53*:376-95, 1959.

6 Colwell, A. R., Jr.: Absence of hypoglycemia after intrapancreatic glucose infusion. Diabetes *12*:437-43, 1963.

7 Colwell, A. R., Jr., Zuckerman, L., and Berger, S.: Pancreatic insulin secretion following intrapancreatic infusion of amino acid. Diabetes *19*:217-27, 1970.

8 Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Diabetes *12*:115-26, 1963.

9 Saifer, A., and Gerstenfeld, S.: The photometric microdetermination of blood glucose and glucose oxidase. J. Lab. Clin. Med. *51*:448-60, 1958.

10 Field, J. B.: Factors concerned with insulin synthesis and release. Metabolism *8*:407-21, 1964.

11 Luft, R.: Some considerations on the pathogenesis of diabetes mellitus. N. Engl. J. Med. *279*:1086-92, 1968.

12 Wright, P. H.: Measurement of insulin secretion. A review of current methods. Diabetes *17*:641-45, 1968.

13 Meade, R. C., Kneubuhler, H. A., Barboriak, J. J., and Schulte, W. J.: Absence of glucose response to physiologic levels of serum insulin. Diabetes *18*:397-401, 1969.

14 Kanazawa, Y., Kuzuya, T., Ide, T., and Kosaka, K.: Plasma insulin responses to glucose in femoral, hepatic and pancreatic veins in dogs. Am. J. Physiol. *211*:442-48, 1966.

15 Colwell, A. R., Jr., and Zuckerman, L.: Regulation of insulin release by pancreatic glucagon. Diabetes *19*:429-37, 1970.

16 Feldman, J. M., and Lebovitz, H. E.: Appraisal of the extrapancreatic actions of sulfonylureas. Arch. Intern. Med. *123*:314-22, 1969.

17 Colwell, A. R., Jr.: Potentiation of insulin action on the liver by tolbutamide. Metabolism *13*:1310-17, 1964.

18 Loubatieres, A., Mariani, M. M., Ribes, G., deMalbosc, H., and Chapal, J.: Etude experimentale d'un nouveau sulfamide hypoglycemiant particulierement actif, le HB 419 ou glibenclamide. Diabetologia *5*:1-10, 1969.