Failure of Prolonged Sulfonylurea Administration to Enhance Insulogenic Response to Glycemic Stimulus

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SUMMARY

The effect of chronic administration of sulfonylurea compounds on the insulogenic responsiveness of beta cells was studied in normal dogs who received hypoglycemia-maintaining dosages of tolbutamide daily for several weeks. Insulin concentration in pancreaticoduodenal venous blood was measured during infusion of glucose at a rate causing physiologic increase in arterial glucose concentration.

Neither fasting plasma insulin level nor its enhancement during progressive hyperglycemia was significantly greater in tolbutamide-treated animals than in the control group.

The data indicate that prolonged exposure of nondiabetic canine beta cells to effective concentrations of sulfonylurea does not increase their insulin-secretory responsiveness to glycemic stimulus. Whether these agents impinge similarly or differently upon diabetic human islets awaits elucidation.

It was parenthetically observed that dogs treated with tolbutamide for several weeks required more than twice the usual amount of Nembutal to maintain surgical anesthesia. DIABETES *14*:392-95, July, 1965.

Acute administration of sulfonylurea compounds stimulates release of preformed insulin, almost immediately from normal beta cells[1,2] and more sluggishly in patients with diabetes mellitus.[2] Whether chronic therapy with these agents also increases the total insulin secretory response to sustained glycemic stimulus has not been reported. In the present study, neither initial insulin release nor total insulin output during continuous hyperglycemia of physiologic magnitude was significantly greater in dogs who had been pretreated for several weeks with tolbutamide.

MATERIALS AND METHODS

Twenty-five mongrel dogs of either sex were used. Animals weighed between 13.2 kg. and 22.3 kg., and all were given the same daily ration of meat and Purina dog chow. Twelve dogs received tolbutamide, the starting dosage of 250 mg. twice daily being increased as necessary to maintain fasting hypoglycemia (table 1). Fasting blood glucose was determined every other day. Duration of tolbutamide therapy varied from thirty-one to eighty-one days, and averaged fifty-seven days. Mean daily dosage per animal ranged from 590 to 1,190 mg., and averaged 760 mg. Based on body weight, daily tolbutamide varied between 35 mg. per kilogram and 90 mg. per kilogram, averaging 49 mg. per kilogram.

TABLE 1

Prolonged administration of tolbutamide to twelve dogs (mean and range)

Body Weight	Daily tolbutamide dosage		Duration	Blood glucose (mg./100 ml.)	
kg.	mg.	mg./kg.	days	Pre-Rx	End of Rx
16.0	760	49	57	66	48
13.2-20.5	590-1,190	35-90	31-81	58-74	29-70

After an overnight fast, the twelve tolbutamide-treated animals and thirteen untreated dogs were laparotomized by midline incision under Nembutal anesthesia (note under "Discussion" the surprising resistance of experimental animals to Nembutal). The free portion of the duodenum was delivered into the wound and reflected to the left in order to expose the cranial pancreaticoduodenal vein draining the right pancreatic limb. The vein was cannulated with a Cournand needle in the direction of blood flow toward the portal vein. Both femoral veins and one femoral artery were also cannulated with Cournand needles. Pancreatic venous blood was drawn into heparinized syringes for determination of plasma insulin by a slight modification of the radioimmunoassay of Yalow and Berson.[3] Chromatographic separation of bound and free insulin-131 in an air-conditioned room (24° C.) was used instead of chromatoelectrophoretic separation in a cold room (4°

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C.). In addition, strips were bisected and counted in a well-type gamma scintillation counter instead of employing a strip scanner. The dose-response curve, the reproducibility of values when the same plasmas were repeatedly assayed, and the range of values obtained in normal and diabetic patients,[2] all duplicated the performance of the original technic. Blood glucose was analyzed by the Somogyi-Nelson method.[4]

After obtaining baseline samples, a solution of 5 per cent glucose was started into one femoral vein at a slow rate controlled by a Bowman infusion pump. Rates of glucose administration were similar in both experimental and control animals, varying from 3.3 mg. per kilogram per minute, to 6.5 mg. per kilogram per minute, and averaging 4.7 mg. per kilogram per minute. A five-minute sample of pancreatic venous blood was obtained for plasma insulin concentration. Thereafter, simultaneous specimens for pancreatic venous insulin, and for femoral arterial and femoral venous blood sugar, were obtained at 10, 20, 30, 45, 60, 80, 100 and 120 minutes. Animals were then killed with Nembutal, and sections of the left limb (tail) of the pancreas were excised and fixed immediately in Bouin's solution. Microscopic sections were stained with Gomori's chromium-hematoxylin-phloxine stain for islet-cell detail.[5]

RESULTS

1. *Changes in arterial blood glucose (table 2 and figure 1).* Blood glucose concentrations were significantly greater in untreated animals, both baseline and during the first twenty minutes of infusion; whereafter the slightly higher mean values in controls became unimportant, and maximal arterial levels were essentially the same in both groups. In control dogs, fasting value of 95 ± 4 mg. per 100 ml. (mean ± S.E.M.) rose steadily to a maximum of 177 ± 9 mg. per 100 ml. at 120 minutes, an increment of 82 ± 7 mg. per 100 ml. In tolbutamide animals, initial blood glucose of 68 ± 5 mg. per 100 ml. ($p < .001$ compared to controls) rose to 171 ± 13 mg. per 100 ml. at 120 minutes. The net increase of 103 ± 16 mg. per 100 ml. was not significantly greater ($p < .3$) than the increment in controls. Arteriovenous glucose differences at corresponding intervals were the same in both groups (data not shown).

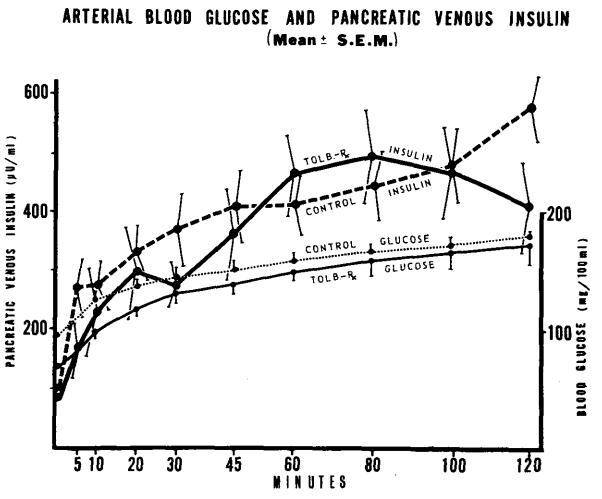

FIG. 1. Baseline plasma insulin concentrations were the same in both control and tolbutamide-treated dogs. Rising arterial glucose elicited proportional enhancement of insulin output in both groups. Differences between corresponding mean plasma insulin values were never statistically significant.

2. *Insulin concentrations in pancreaticoduodenal venous blood (table 2 and figure 1).* Mean plasma in-

TABLE 2

Arterial blood glucose and pancreatic-venous plasma insulin during infusion of glucose (Mean ± S.E.M.)

	0 Min.	5 Min.	10 Min.	20 Min.	30 Min.	45 Min.	60 Min.	80 Min.	100 Min.	120 Min.
			BLOOD GLUCOSE (mg. per 100 ml.)							
Control Dogs (13)	95 ±4		124 ±7	134 ±6	143 ±7	150 ±7	158 ±8	166 ±8	170 ±8	177 ±9
Tolbutamide Dogs (12)	68 ±5		97 ±5	115 ±6	130 ±7	137 ±7	149 ±8	156 ±10	166 ±12	171 ±13
"p"	<.001*		<.01	<.05	<.2	<.2	<.5	<.5	<.8	<.8
			PLASMA INSULIN (microunits per ml.)							
Control Dogs	95 ±10	269 ±49	271 ±32	325 ±43	363 ±61	402 ±67	404 ±57	439 ±60	471 ±64	578 ±58
Tolbutamide Dogs	83 ±16	163 ±50	220 ±82	295 ±78	278 ±39	358 ±78	460 ±73	491 ±78	468 ±72	409 ±75
"p"	<.6*	<.2	<.6	<.8	<.3	<.7	<.6	<.7	>.9	<.1

* "p" compares corresponding values for control and tolbutamide-treated groups.

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sulin levels were actually lower at most sampling periods in tolbutamide-treated dogs, and never differed significantly from corresponding control values. In untreated animals, plasma insulin almost tripled immediately, from fasting value of 95 ± 10 microunits per milliliter to 269 ± 49 microunits per milliliter at five minutes, and increased along with rising arterial glucose to a peak value of 578 ± 58 microunits per milliliter at 120 minutes. In tolbutamide-treated dogs, mean fasting plasma insulin of 83 ± 16 microunits per milliliter ($p < .6$ compared to controls) had doubled to 163 ± 50 microunits per milliliter in five minutes, continued climbing to a peak concentration of 491 ± 78 microunits per milliliter at eighty minutes, and gradually declined thereafter.

3. *Microscopic appearance of islets of Langerhans*: Both the total number of islets and the size of individual islets in the left limb (tail) of the pancreas were indistinguishable in treated versus untreated animals. Moreover, differential Gomori staining revealed approximately the same proportion of beta cells in islets of each group.

DISCUSSION

By direct measurement, the present data show that prolonged and continuous stimulation of normal beta cells by sulfonylurea compounds does not enhance their insulogenic responsiveness. These results support the senior author's previous interpretation, upon finding prompt but transient rise in pancreatic venous insulin-activity after acute administration of sulfonylureas, that these agents release preformed insulin but do not engender new insulin formation.[1] In a sense, therefore, they are "inferior" to the physiologic glycemic stimulus, which not only releases stored insulin but also activates synthesis of insulin de novo. On the other hand, this passive role of merely "solubilizing" stored insulin is an acceptable limitation of potency, since it may explain why years of continuous administration of these agents to elderly diabetics does not exhaust beta cell reserve and accelerate breakdown of carbohydrate tolerance.

A short-term, permissive effect of sulfonylureas is similarly suggested by available clinical knowledge. In both normal subjects and mild diabetics, administration of these agents for several days lowers fasting blood glucose level without improving the oral glucose tolerance curve.[6] Moreover, although prolonged sulfonylurea therapy sometimes does restore normal carbohydrate turnover in patients with mild diabetes, this improvement apparently requires maintenance of effective blood concentrations of sulfonylurea. Even after years of successful management, Fajans[7] saw glucose tolerance deteriorate toward pretreatment status within days after stopping tolbutamide. Such observations suggest that the important metabolic effect of these compounds is to lower the concentration of glucose in pancreatic arterial blood at which mildly diabetic beta cells release stored insulin. The clinical sequelae of this single action might reasonably be both reduction of the fasting blood glucose level and restoration of the normal swift insulogenic response to a rising blood sugar level.[1,2]

It was parenthetically found that dogs treated with tolbutamide for several weeks were amazingly resistant to Nembutal anesthesia, both for initial induction and for maintaining the anesthetized state. As shown in table 3, induction dosage in control dogs averaged 28 ± 1 mg. per kilogram, and total dosage 37 ± 2 mg. per kilogram; whereas tolbutamide-treated animals required an induction dosage of 38 ± 2 mg. per kilogram ($p < .001$), and a total of 86 ± 7 mg. per kilogram ($p < .001$). The pharmacologic explanation for this phenomenal tolerance to barbiturate is obscure. In addition, since the dog metabolizes tolbutamide by cleaving the butyl side chain,[8] whereas man oxidizes it to a carboxylic acid derivative,[9] the clinical relevance of this observation is likewise unknown.

TABLE 3

Resistance of tolbutamide-treated dogs to Nembutal anesthesia

	Dosage (mg. per kg.—mean ± S.E.M.)	
Group	Induction	Total
Control	28 ± 1	37 ± 2
Tolbutamide	38 ± 2	86 ± 7
"P"	< .001	< .001

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Diet and Human Depot Fat

Fatty acids of depot fat represent those eaten or synthesized minus those excreted or degraded. Evidence has been presented from studies with man indicating that the pattern of fatty acids in the depot fats can be altered by changes in diet. Unless the diet change is extreme, alterations appear to take place slowly over a period of months or years. One might infer from this that the depot fat is relatively stable. However, a study by K. J. Kingsbury et al. (Biochem. J. 84:124, 1962) suggests the possibility that the fatty acid composition of the depots instead of being stable may be in a continual state of flux, often without apparent relation to the dietary intake.

Eight male students aged nineteen to twenty-three with no history of any lipid abnormality of liver or of pancreatic, kidney or thyroid disease were used as experimental subjects.

From the over-all data the authors conclude: that the dietary polyunsaturated acids appear readily in plasma lipids but only sparsely in depot fats; that marked individual changes occur in the monoene unsaturated fatty acids of the plasma and depot fat apparently unrelated to the type of dietary lipid; that the fatty acid changes of the plasma and depot fat are often dissimilar; that the fatty acid composition of the body is in a continual state of flux, often unrelated to dietary fat; and that changes in the lipids of one body compartment are not reliable indicators of changes in another.

Studies of fatty acids in blood and depot fat require a series of complex, time-consuming procedures and Kingsbury and co-workers should be commended for the intensive work they have done. However, the number of individuals (which in several important instances is only two) on which the conclusions are based, would appear to be too small to warrant definitive statements. In regard to the plasma fatty acids, one would expect a sensitive response to feeding of those polyunsaturated fats not readily synthesized in the body and a less sensitive response to the saturates and monoenes that are readily synthesized, and this is essentially what was observed.

The report of Kingsbury et al. points up the elementary state of our knowledge of fat metabolism in man, even in regard to information requiring relatively simple observations. Further studies should be done including some involving multiple biopsies of adipose tissue from the same individual which should resolve the problem of the stability of the fatty acids of adipose tissue.

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