Studies Concerning the Hyperglycemic Effects of Diazoxide and Its Mode of Action

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SUMMARY

Diazoxide in doses of 200 mg. per kg. was administered orally to normal, adrenalectomized, hypophysectomized, and partially pancreatectomized rats. The hyperglycemic effect was studied five hours after drug administration.

It was observed that: (1) Diazoxide produced no hyperglycemia in adrenalectomized animals. (2) Diazoxide hyperglycemia was lower in hypophysectomized animals, disappearing entirely one month after hypophysectomy. (3) Diazoxide hyperglycemia in partially pancreatectomized animals diminished with prolonged drug administration over a period of twenty-two days.

These experiments in partially pancreatectomized rats are being continued. DIABETES *14*:591-94, September 1965.

It has been reported that diazoxide is a useful substance in a number of hypoglycemic states, Drash et al.[1] Frerichs et al.[2] reported that diazoxide inhibited insulin release in rat pancreas incubated in vitro, insulin activity being measured by the epididymal fat pad technic.

The usefulness of diazoxide as a hyperglycemic agent, as well as the light its use might throw on the mechanisms of insulin release, has led to the present studies concerning the mechanism of its hyperglycemic activity. There is no experimental proof of permanent hyperglycemia resulting from previous diazoxide treatment.

It was the purpose of the present study to elucidate the part played by the adrenal, pituitary, and pancreas in diazoxide hyperglycemia.

MATERIAL AND METHODS

Animals used in all experiments were male albino rats of the CFE strain as supplied by Carworth Farms. The animals were maintained on a standard (Wayne Lab-Blox) diet, given ad libitum. The weight and number of the animals are specified in each particular group (see Results).

Adrenalectomized animals: Adrenalectomy was performed in our laboratory. Both adrenals were removed through a single posterior incision under ether anesthesia. After the operation the animals were kept on 1 per cent sodium chloride in drinking water ad libitum. Diazoxide was given seventy-two hours after adrenalectomy.

Hypophysectomized animals: They were obtained from commercial source and experimented on seventy-two hours after hypophysectomy and again one month later. One per cent sodium chloride was added to drinking water fifteen days after operation.

Pancreatectomized animals: Partial pancreatectomy (95 per cent of pancreas removed), was accomplished following the technic described by Foglia;[3] animals were taken for experiment eight days after operation.

Diazoxide (200 mg. per kg. of body weight) was administered in all cases by a stomach tube. An aqueous suspension of 2 gm. of the drug was solubilized in 10 ml. of 1 N sodium hydroxide, diluted with distilled water to approximately 80 ml., retitrated with 1 N hydrochloric acid (approximately 1.8 to 1.9 ml.) to a *p*H of 10.5 and made up to 100 ml. with distilled water. One milliliter of this solution containing 20 mg. of diazoxide was given for each 100 gm. of body weight. The solvent used in control animals was of the same composition, but did not contain diazoxide and was also given by a stomach tube.

Levels of blood sugars were estimated in 0.1 ml. of whole blood obtained from the tip of the tail before and five hours after the drug administration, using the Technicon AutoAnalyzer and the ferricyanide micromethod.[4] Occasional spot-check determinations were performed by the Nelson-Somogyi method[5] in our own modification suitable for microdetermination.

Glucose tolerance was estimated following the administration of 3 gm. of glucose per kg. of body weight (in a 30 per cent solution), by tube. Blood sugars were measured before, and thirty, ninety and 150 minutes after glucose feeding.

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TABLE 1

Effect of diazoxide (200 mg. per kg.) on the blood sugar in normal adrenalectomized and hypophysectomized rats

	Normal rats				Adrenalectomized rats 72 hours after operation				Hypophysectomized rats 72 hours after operation				Hypophysectomized rats 1 month after operation			
	Placebo		Diazoxide		Placebo		Diazoxide		Placebo		Diazoxide		Placebo		Diazoxide	
Time*	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5
Number of animals	10	10	9	10	10	10	10	10	11	11	10	14	9	8	8	8
Average weight (gm.)	300	300	300	300	295	295	279	279	140	140	127	127	177	177	152	152
Blood sugar mg. per 100 ml. (average)	118	104	117	146	93	80	96	82	111	93	117	121	103	90	102	95
Average change of blood sugar in five hours (mg. per 100 ml.)	−14±9		+29±7		−13±2		−14±3		−18±4		+4±4		−13±5		−7±6	
Significance of the five-hour blood sugar change	Not significant		Increase P<.01		Decrease P<.01		Decrease P<.01		Decrease P<.01		Not significant		Decrease P<.02		Not significant	
Statistical comparisons Placebo vs. diazoxide	P<.01				Not significant				P<.01				Not significant			

*0 time = before treatment
5 time = five hours after drug administration

Ascorbic acid estimation in adrenal tissue was done in metaphosphoric-acetic acid extract of tissue homogenates by a modified 2,4-dinitrophenylhydrazine method of Roe and Kuether.[6]

Statistical significance of the results was established using the Student's *t*-test.

RESULTS

A. Table 1 shows the blood sugar response to a single dose of diazoxide in normal, adrenalectomized and hypophysectomized animals.

It can be seen that the five-hour hyperglycemic effect of diazoxide observed in normal rats is not present in adrenalectomized rats, while it persists, although greatly diminished, in the hypophysectomized animals (seventy-two hours after operation). This diminished effect in hypophysectomized animals, however, disappears entirely one month after hypophysectomy.

The ascorbic acid content of the adrenals was estimated five hours after oral diazoxide administration (200 mg. per kg.) in normal rats. Five animals received diazoxide and the ascorbic acid content was 379 mg. per cent. Nine received placebos under the same conditions, and the ascorbic acid content was 523 mg. per cent ($P < 0.1$).

B. On the first day after operation diazoxide had a greater response in the 95 per cent pancreatectomized rat than in the normal rat. Prolonged treatment of normal rats with diazoxide shows that the hyperglycemic effect remained constant during the twenty-two days of the experiment, while in partially pancreatectomized rats there was a gradual decrease of the hyperglycemic response during the same time period (table 2, figure 1).

This gradual decrease was tested by an analysis of variance showing significance ($F\ 2, 33 = 4.21\ P < .025$).

Immediately after the twenty-second day, the diazoxide administration was interrupted and an oral glucose tolerance test was performed seventy-two hours after the last dose of diazoxide. Table 3 suggests that the group of pancreatectomized rats that were treated with diazoxide had an improved glucose tolerance compared to the nontreated placebo group.

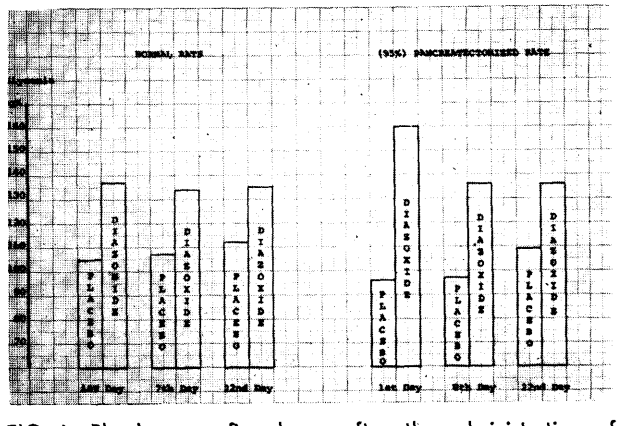

FIG. 1. Blood sugar five hours after the administration of 200 mg. per kilogram of diazoxide (oral) during twenty-two days.

TABLE 2

Effect of diazoxide (200 mg. per kg.) on blood sugar in pancreatectomized (95 per cent) rats during twenty-two days of treatment

Days of treatment	1		8		22	
	Placebo	Diazoxide	Placebo	Diazoxide	Placebo	Diazoxide
Number of rats	14	13	13	12	14	12
Average weights	154	146	184	172	228	218
Blood sugar average (mg. per 100 ml.)	96±3	160±7	97±3	136±6	109±2	136±5
Statistical comparisons						
Placebo vs. diazoxide	P<.001		P<.001		P<.001	

*Blood sugar five hours after drug administration

TABLE 3

Oral glucose tolerance test in pancreatectomized rats after twenty-two days of diazoxide treatment*

	Pancreatectomized (95 per cent) rats				
	Number of rats	Minutes after glucose administration 0	30	90	150
Control	14	115±3	153±6	151±7	116±3
Diazoxide	12	115±2	142±6	132±4†	117±2
		Blood sugar in mg. per 100 ml.			

*Diazoxide treatment is interrupted seventy-two hours before the test.
†Significant P < .05

DISCUSSION

Previous work, Dollery et al.,[7] and Wolff et al.,[8] suggests the possibility that diabetes might be produced by treatment with diazoxide in normal or so-called prediabetic patients. Our results confirm that diazoxide is a hyperglycemic substance, but that under the conditions of our experiment this effect is transitory and disappears in a few hours. It is also apparent that this hyperglycemia is at least partially mediated by intact adrenal glands. Those results confirm those reported by Tabachnick et al.[9]

It is of interest that the recently 95 per cent pancreatectomized rat has a greater hyperglycemic response to diazoxide than the normal rat. Young, 150 gm. average weight rats were used for this experiment, and it suggests that in this "prediabetic" situation diazoxide may act as a stressful factor, anticipating the spontaneous development of diabetes expected later on.[3]

The possibility of pancreatic damage from diazoxide seems to be ruled out by the fact that, as time went on, partially pancreatectomized rats receiving diazoxide developed neither hyperglycemia nor permanent diabetes. The untreated control group of similarly pancreatectomized animals remained normoglycemic during the period of observation.

Foglia[3] has demonstrated that rats with 95 per cent pancreatectomy go through a period of normoglycemia for about three months. After that, abnormal glucose tolerance and glycosuria appear and the rats become rapidly diabetic. This period of normality after partial pancreatectomy has been called prediabetic. Many drugs and hormones have been administered during this prediabetic period in order to prove their protective or deleterious effect. Diazoxide used in our experiments shows no deleterious effect, but on the contrary, possibly a protective influence from the eighth to the twenty-fifth day of drug administration. Prolonged administration of glucose, corticosteroids and estrogens to partially pancreatectomized rats has been reported to have a protective influence.[10-14] It has been suggested that prolonged hyperglycemic activity stimulates beta cell regeneration.

On the other hand the improvements in glucose tolerance obtained in our experiments on partially pancreatectomized rats could be the consequence of adrenal stimulation. In an acute experiment there was depletion of ascorbic acid in the adrenals following diazoxide, suggesting increased adrenal activity.

Adrenal participation in the hyperglycemic response to diazoxide appears confirmed as the adrenalectomized rats showed no hyperglycemia following diazoxide administration. Liver glycogen stores seventy-two hours after adrenalectomy were not depleted, nor was there a diminution of the hyperglycemic effect of diazoxide in the fasted normal rat.* Hypophysectomized rats showed less hyperglycemic effect following diazoxide immediately after hypophysectomy (when the adrenals were still functioning to some extent) but no effect could be demonstrated after a longer period of time (adrenal activity greatly diminished).

*Unpublished observations from this laboratory, in preparation for publication.

Other experiments are in progress to elucidate the possible mode of action of the adrenals in diazoxide hyperglycemia.

No diabetes developed in normal or partially pancreatectomized rats after twenty-two days of treatment with diazoxide. The glucose tolerance tests in these animals improved after prolonged diazoxide administration, though only the data at the ninety-minute level reached statistical significance. These tests are being continued for a longer period of time.

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