Stimulation of Pancreatic Islet Cells by 2-Deoxy-D-Glucose.* (26535)

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2-Deoxy-D-Glucose (2-DG) inhibits normal utilization of glucose in the cell and leads to cellular glucopenia(1). In intact animals this condition is accompanied by marked adrenaline release which seems to play a major role in induction of hyperglycemia by 2-DG(2). The present study investigated whether 2-DG affects the alpha and beta cells of the pancreatic islets, the functions of which are intimately related to glucose metabolism.

Material and methods. The experiments involved white inbred male rats, 175-275 g, maintained on standard diet. During acute experiments no food was given, but free access to water was allowed. 2-DG† was dissolved in water, 50 mg/ml, and administered subcutaneously. In acute experiments the single injection (50 mg/100 g b.w.) was given at 8 a.m. In long term experiments the same dose was injected at 8 a.m. and 7 p.m. for 6 days. Control animals received a corresponding quantity of distilled water. In acute experiments glucose in tail blood was determined at different times following 2-DG, in long term experiments only prior to and one hour after the first injection on days 1 and 6. Glucose was determined colorimetrically using condensation with *o*-toluidine(3), a method in which 2-DG is only one-tenth as chromogenic as glucose(2). The animals were killed by decapitation at various times after 2-DG, the experiments being arranged so that all rats were killed about 8:00 p.m.

Pancreatic islet cell activity was evaluated on basis of changes in stainability, granulation and nuclear size. The pancreas was fixed in Bouin's fluid and stained with chromhaematoxylin-Ponceaufuchsin or with paraldehydefuchsin-Ponceaufuchsin. Nuclear size was determined with a planimeter using section 7 μ thick; about 50-70 alpha and 50-70 beta nuclei were traced off at a magnification of approximately 2,000. Under these conditions the standard error of a single determination in percentage of ordinary mean values is 2.1-3.0 for alpha cells and 1.1-1.5 for beta cells (4).

Results. Pronounced hyperglycemia appeared as early as 30 minutes after administration of 2-DG (50 mg/100 g b.w.) and lasted for at least another 4-6 hours. Blood sugar approached control values at 12 hours (Table I). During this period marked cytological changes appeared in beta and alpha cells. There was enlargement of beta cell nuclei, highly significant 6 hours after 2-DG and probably significant after 9 hours (Table I). Concomitantly stainability and granulation of the cytoplasm decreased (Fig. 1 and 2), particularly in the central parts of the islets. After 12 hours all these parameters had become normal.

In the alpha cells cytological changes appeared analogous to those described for the beta cells. Although not statistically significant until 6 hours after injection of 2-DG, the alpha cell nuclei seemed enlarged after 1-3 hours; at 9 hours nuclear size was still increased (probably significant) while at 12 hours no difference could be demonstrated as compared to controls (Table I). The decreased stainability and degranulation of the cytoplasm of the alpha cells is shown in Fig. 3 and 4. In those alpha cells presenting the most marked nuclear changes, vacuolation was visible. Furthermore, the nuclear bodies stained intensely in 2-DG-treated animals.

In long term experiments blood sugar rose from 81 $\pm$ 2.7 mg% to 207 $\pm$ 11.4 one hour after first injection on day 1, the corresponding figures for day 6 being 78 $\pm$ 2.1 and 160 $\pm$ 9.2. The average size of the beta cell nuclei was 29.6 μ^2 in treated rats, as com-

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TABLE I. Blood Glucose and Nuclear Area of Alpha and Beta Cells Following a Single Injection of 2-DG.

Time after inj., hr	Blood glucose, mg % ± S.E. Controls	2-DG, 50 mg/100 g b.w.	Alpha cells Mean value, μ^2	P	Beta cells Mean value, μ^2	P
0	88 ± 1.7	84 ± 2.6	22.0*		25.5*	
½	—	200 ± 6.6				
1	92 ± 3.9	271 ± 8.0	23.3	>.05	26.1	>.05
2	90 ± 3.4	242 ± 6.2				
3	91 ± 3.7	—	23.7	>.05	25.8	>.1
4	88 ± 1.5	195 ± 8.1				
6	90 ± 4.0	122 ± 5.1	25.2	<.01	27.8	<.001
9	91 ± 4.1	110 ± 3.5	24.1	<.02	29.2	<.05
12	92 ± 4.6	103 ± 4.1	22.3	>.1	25.7	>.1

* Non-inj. controls.

P = probability.

Fig. 1 Fig. 2

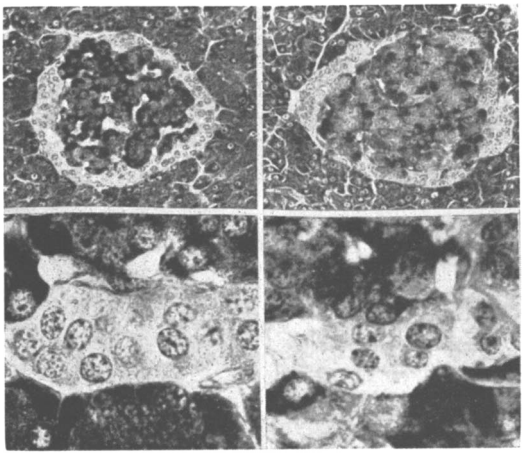

Fig. 3 Fig. 4

FIG. 1. Islet in a control rat. Paraldehydefuchsin-Ponceaufuchsin. × 180.

FIG. 2. Islet 9 hr after 2-DG. Reduced stainability in beta cells most pronounced in central parts of islet. Same stain and magnification as in Fig. 1.

FIG. 3. Group of alpha cells in an islet of a control rat. Same stain as in Fig. 1. × 600.

FIG. 4. Group of alpha cells showing reduced stainability and granulation with vacuolisation following 2-DG. Same stain and magnification as in Fig. 3.

pared to 26.0 in controls (P = <0.01). For the alpha cells the corresponding values were 24.6 μ^2 and 22.3 μ^2 respectively (P = 0.01). By mistake 2 control rats were given 2-DG twice on the day prior to termination of the experiment, possibly causing some enlargement of the pancreatic islet cell nuclei in the control group. Signs of degeneration of alpha or beta cells were not noted, either in acute or in long term experiments. Increased mitotic activity was not observed.

Discussion. The combination of nuclear enlargement, degranulation, and decreased stainability of the cytoplasm in the beta cells of the pancreatic islets probably represents increased activity(5). While increased activity of the beta cells is generally considered to result in insulin secretion, the interpretation of similar changes in the alpha cells is disputed. It seems likely that these changes in the alpha cells also reflect stimulation, and there are reasons to believe that alpha cells produce glucagon(6,7). Our findings may then indicate that 2-DG may cause increased secretion of both insulin and glucagon.

With respect to the beta cells, there is general agreement that hyperglycemia stimulates insulin secretion, and it seems likely that this was the effective stimulus in our experiments. On the other hand, insulin produced hypoglycemia stimulates the alpha cells(8), and it seems paradoxical to find evidence of alpha cell stimulation in the presence of hyperglycemia. However, 2-DG imposes cellular glucopenia and it is reasonable to assume that this is the effective stimulus for the alpha cells. It may be that an identical mechanism is involved in alpha cell stimulation following both insulin and 2-DG. Similarly, the centrally located receptor system regulating adrenaline secretion has been found to be stimulated by either insulin or 2-DG.

Summary. In the intact rat 2-Deoxy-D-Glucose (2-DG) leads to decreased stainability and degranulation of the cytoplasm and increased nuclear size of both alpha and beta cells of the pancreatic islets. Maximal changes occurred 6 hours after administration of 2-

DG. The findings are interpreted to indicate increased activity in both types of cells and the underlying regulatory mechanisms are discussed.

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Differential Anaphylactic Susceptibility of Chicken and Guinea Pig Intestine.* (26536)

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The possibility of using the intestine of chickens, instead of that of guinea pigs, in the Schultz-Dale reaction was investigated. Since we were unable passively to sensitize the chicken intestine, a study was made of factors that might contribute to the differential anaphylactic susceptibilities of chickens and guinea pigs.

Materials and methods. White Leghorn chickens, weighing 1.3 to 1.7 kg, were given a single intravenous sensitizing injection of chicken or rabbit antiserum containing 25 mg of antibovine serum albumin (anti-BSA) antibody per kg body weight. Both antiserums contained 1.5 mg of precipitating antibody protein per ml of serum as estimated by the quantitative precipitin test (8% salt concentration used for chicken serums)(1). Chickens were fasted for 16 to 18 hours, killed by a blow on the head, and 15 cm of the lower portion of the small intestine cranial and adjacent to the caecum was carefully removed and kept moistened at all times with modified Tyrode solution(2). The intestinal contents were carefully removed by flushing the intestine with 20 ml of fresh Tyrode solution. For each experiment, one control and one experimental strip, 4 cm in length, were immersed in a 120 ml muscle bath of the Schultz-Dale apparatus(3) containing Tyrode solution at 40°C and a stream of O^2 (90%) plus CO_2 (10%) was used to aerate and stir the bath. The intestinal strips were allowed to equilibrate in the bath for 15 minutes, then their reactions were tested by adding 9 mg of BSA, 25 to 800 μg (in 2-fold increments) of histamine, and 0.1 to 0.8 μg (in 2-fold increments) of acetylcholine chloride. After each test the bath was drained, the intestinal strips washed, and fresh Tyrode solution added and equilibrated for 8 minutes.

Essentially the same procedure was followed for the guinea pig. Guinea pigs weighing 0.4 to 1 kg were divided into 5 groups. The first 2 groups received 0.25 and 0.5 mg of rabbit anti-BSA serum per kg body weight, respectively, and the third group received 25 mg of chicken anti-BSA serum per kg body weight. The other 2 groups, which served as controls, were injected with normal rabbit and chicken serums, respectively. The contractility of the ileum, 4 cm in length, was tested with 9 mg of BSA, 0.025 to 0.2 μg (in 2-fold increments) of histamine, and 0.01 to 0.08 μg (in 2-fold increments of acetylcholine in the Schultz-Dale bath maintained at 38°C.

Results. As indicated in Table I, 25 mg of either rabbit or chicken antibody per kg

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