ZINC AND INSULIN CONTENT IN PANCREATIC ISLETS OF RATS AFTER ADMINISTRATION OF HYPOGLYCEMIC SULFONAMIDES

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Hypoglycemic sulfonamides of the original (chlorpropamide, tolbutamide) and more recent (euglucon-5) phases of development were administered to rats by the intragastric route. Euglucon-5 induces marked hypoglycemia in doses 50-100 times smaller than chlorpropamide and tolbutamide. All these substances can expel zinc and insulin from the β cells of the pancreatic islets. However, the ability of euglucon-5 to mobilize zinc and insulin is much greater than that of chlorpropamide and tolbutamide. The fact that the changes in the zinc and insulin content in the islet tissue are identical in direction is evidence of their close functional relationship.

Zinc is found in the pancreatic islets of many animals and man [1]. Zinc is considered to form an insoluble complex [10, 11] with insulin in the cytoplasm of the β cells, in which it occurs in the secretory granules, the number of which reflects the insulin content in the islets [8]. During glucose loading the number of granules in the β cells decreases in accordance with their loss of insulin [9]. Degranulation of these cells can be observed experimentally after administration of hypoglycemic sulfonamides to the animals. Under these circumstances the disappearance of insulin from the β cells is accompanied by loss of their zinc [3, 4, 6].

The content of insulin and zinc was investigated in the pancreatic islets of rats after administration of hypoglycemic sulfonamides of the original (chlorpropamide, tolbutamide) and more recent (euglucon-5) phases of their pharmacological development.

EXPERIMENTAL METHOD

Experiments were carried out on 43 noninbred male albino rats weighing 200-240 g. Chlorpropamide (0.2-2.3 g/kg), tolbutamide (2.2-2.5 g/kg), and euglucon-5 (25-150 mg/kg) were administered by gastric tube as a suspension in liquid starch emulsion. The blood sugar was determined by the Hagedorn–Jensen method. The animals were killed with ether 24 h after receiving the test compounds. Pieces of the pancreas measuring 2-3 mm were fixed in 70% ethanol saturated with hydrogen sulfide for 4-5 h at a low temperature. The dewaxed sections of the gland were stained with 1% methylene blue. The appearance of dark brown granules of reduced silver in the pancreatic islets indicated that they contained heavy metals. Part of the gland was fixed in Bouin's fluid. The dewaxed sections were stained with aldehyde-fuchsin. The granules thereby revealed in the β cells correspond to the reserve form of insulin. The insulin content also was judged by the highly sensitive metachromatic reaction with pseudoisocyanin [5, 7, 12].

EXPERIMENTAL RESULTS

Severe hypoglycemia (Table 1) developed in all the rats 24 h after receiving euglucon-5, but its level did not correlate with the dose of the compound. Administration of 200 mg/kg chlorpropamide was fol-

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TABLE 1. Effect of Original and More Recent Sulfonamide Preparations on Blood Sugar Level in Rats 24 h after Administration (M ± m)

Compound administered	No. of rats	Dose of compound	Blood sugar (in mg %)		P
			before adminis.	after adminis.	
Control	10		113±2,7	—	
Euglucon-5	4	25—150 mg/kg	109±6,0	56±5,3	<0,001
Chlorpropamide	2	200 mg/kg	136	109	
"	4	1,14—2,3 g/kg	109±2,9	62±7,2	<0,001
Tolbutamide	4	2,2—2,5 g/kg	121±10,0	67±4,3	<0,001

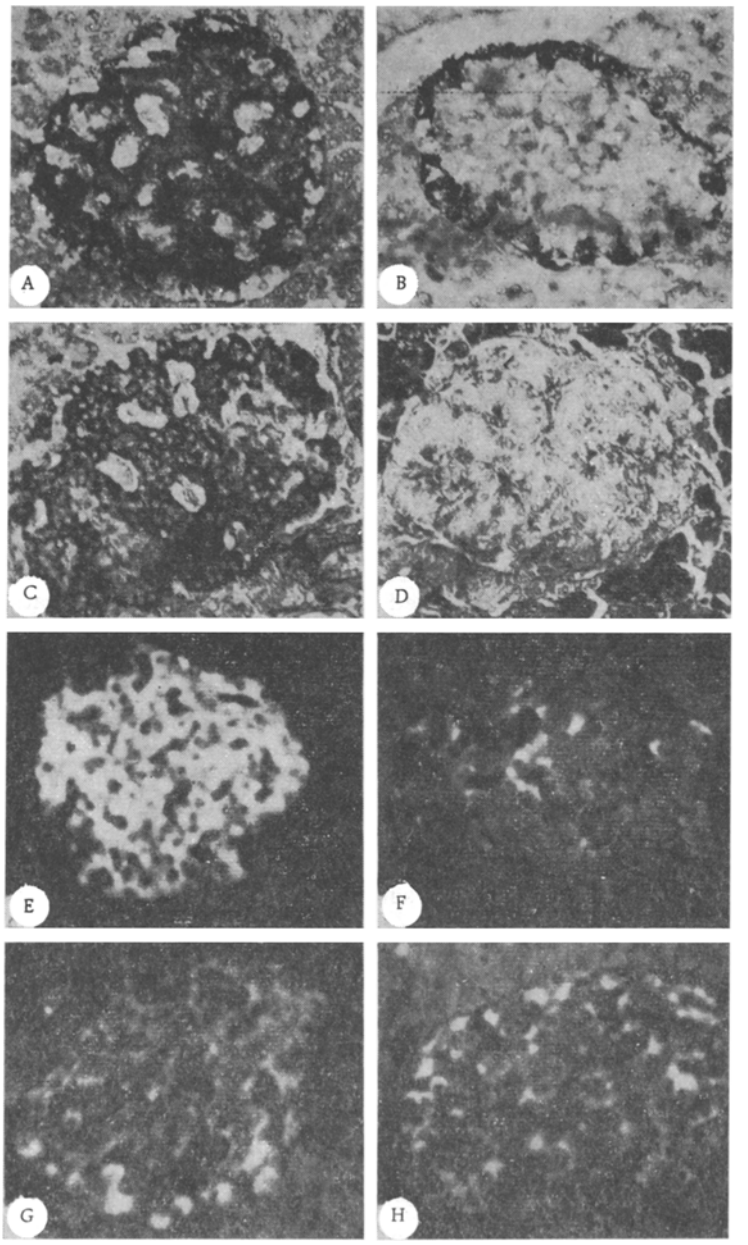

Fig. 1. Histochemical reactions with zinc and insulin in the pancreatic islets: A) zinc in islet of control rat, silver-sulfide method, 200×; B) islet after administration of euglucon-5, silver-sulfide method, 200×; C) islet of control rat, aldehyde-fuchsin, 200×; D) islet after administration of euglucon-5, aldehyde-fuchsin, 200×; E) insulin in islet of control rat, pseudoisocyanin, 120×; F) islet after administration of euglucon-5, pseudoisocyanin, 120×; G) islet after administration of chlorpropamide, pseudoisocyanin, 120×; H) islet after administration of tolbutamide, pseudoisocyanin, 120×.

lowed by only a slight decrease in the blood sugar. Larger doses of this compound (1.14-2.3 g/kg) led to marked hypoglycemia. A sharp decrease in the blood sugar level also was observed in the rat 24 h after receiving large doses of tolbutamide.

All the compounds tested thus had a marked hypoglycemic action in healthy rats. Euglucon-5 led to marked hypoglycemia in doses 50-100 times smaller than chlorpropamide and tolbutamide.

In healthy rats most zinc is localized in the α cells of the pancreatic islets [13]. In sections through the pancreas stained by the silver-sulfide method the α cells stained deep black and the β cells dark brown, almost black (Fig. 1A). Numerous dark purple granules were revealed in the β cells by the aldehyde-fuchsin method (Fig. 1C). After staining with pseudoisocyanin a bright orange luminescence was seen where insulin was present (Fig. 1E).

Administration of euglucon-5 in small doses (25 and 50 mg/kg) led to an appreciable but not total loss of metal from the islet cells. An increase in the dose to 100 and 150 mg/kg led after 24 h to the almost total loss of zinc from the β cells. Its content in the α cells was unchanged (Fig. 1B). Simultaneously with the loss of zinc most of the β cells also lost their aldehyde-fuchsinophilic granules (Fig. 1D). The islets did not stain with pseudoisocyanin. Only isolated β cells still contained a little luminescent substance located mainly at the periphery (Fig. 1F). The elimination of zinc and insulin from the islet cells under the influence of euglucon-5 thus depends on the dosage of the compound.

After administration of 200 mg/kg chlorpropamide there was no visible loss of zinc or insulin from the β cells. Chlorpropamide and tolbutamide had a similar action in large doses. After 24 h most of the β cells had lost their zinc and only a few still contained some of thier silver sulfide granules. The content of the metal in the α cells was unchanged. The aldehyde-fuchsinophilic granules had disappeared from most of the β cells. Insulin was revealed by pseudoisocyanin likewise in only a few cells (Fig. 1G, H).

All three compounds studied thus have a similar action on the pancreatic islet cells of healthy rats. However, the ability of the more recent compound, euglucon-5, to lower the blood sugar and to eliminate zinc and insulin from the β cells is much stronger than that of chlorpropamide and tolbutamide. The fact that the changes in the intensity of the reactions for zinc and insulin take place simultaneously is evidence that they are closely linked functionally.

The results agree with what little information is at present available to show that hypoglycemic sulfonamides stimulate the liberation of zinc and of zinc-bound insulin from the insular β cells [3, 4, 6]. They do not confirm the findings of those workers who state that tolbutamide lowers the insulin level in the islet-cell tissue but at the same time increases its zinc content [2].

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