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Pharmacologic Differentiation Between Epinephrine- and HGF-Hyperglycemias: Application in Analysis of Cobalt-Hyperglycemia.*† (20653)

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Considerable interest has centered upon the hyperglycemic-glycogenolytic factor (HGF) and its putative role as a hormone. Since HGF is found in association with the alpha-cells of the Islets of Langerhans, definitive tests for a true hormonal role of HGF should be greatly simplified by the recent observations(1,2) that cobaltous chloride selectively damages the alpha-cells. Contrariwise, the similarity in action of HGF and epinephrine (and sympathetic nerve activity) is so pronounced that methods must be at hand to distinguish between these hyperglycemic agents. The hyperglycemia produced by each of these agents is related to an activation of hepatic phosphorylase activity(3). Conditions which interfere with the action of HGF affect equally the action of epinephrine(3,4). Our observation that the adrenergic-blocking drug dihydroergotamine (DHE) prevents the effect of epinephrine on liver slices, but does not interfere with the effect of HGF, has led to a practical differentiation of these two hyperglycemic agents *in vivo*.

This paper presents evidence for the selective blockade of the action of epinephrine on glucose liberation by liver slices and on blood sugar of the intact rabbit, under conditions which allow HGF to produce its usual effects. The information obtained on the intact rabbit has been applied to determine the mechanism of the hyperglycemia which follows the administration of cobaltous chloride, an agent which selectively damages the cells in which HGF is found. The maintenance of normal blood sugar level in rabbits was not influenced by the conjoint effects of damaging the alpha-cells with cobaltous chloride, fasting, and subsequent treatment with DHE.

Methods. Rabbit liver slices were tested as described by Sutherland and Cori(5). The tissues were usually incubated with DHE for 30 minutes before addition of the glycogenolytic agents. In a few cases DHE itself produced a small but significant glycogenolytic effect. For *in vivo* studies rabbits were fasted 20-24 hours before tests. Blood samples were obtained from the marginal ear veins. Glucose determinations were made *ad modum*

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TABLE I. Effects of DHE on the Epinephrine- and HGF-Activation of Glucose Production by Rabbit-Liver-Slices.

1 ml 0.2 ml	Sal. P. Sal. P.	Sal. P. EPI	DHE Sal. P.	Sal. P. Epi	DHE Epi	Sal. P. HGF	DHE HGF
			mg glucose/g/90 min.				
Exp. 1	10	28	15	24	12	21	19
2	9	25	9	22	17	21	21

Rabbit-liver-slices were incubated 90 min., in O_2, at 37°C, in mixture of 4 parts 0.9% NaCl and 1 part 0.1 M sodium phosphate buffer at pH 7.4 (Sal. P.). During first 30 min. the slices were incubated in 1 ml of either Sal. P. or Sal. P. containing 1.2:100000 DHE, and then 0.2 ml of Sal. P., or of l-epinephrine bitartrate (1:100000, EPI; 1:1000000, Epi) or of 1:50000 HGF were added as indicated in table. The higher concentration of epinephrine elicited a maximal response which would also be observed with higher concentrations of HGF. Figures are averages of duplicate experiments.

TABLE II. Effects of DHE on Epinephrine- and HGF-Hyperglycemias.

		Avg blood sugar (mg %) ± S.E.			
		Time before		Time after hyperglycemic agent	
	No. of animals	−30′	0′	30′	60′
Epinephrine	8		101 ± 1	144 ± 7	139 ± 5
DHE + epinephrine	7	101 ± 2	105 ± 3	111 ± 7	113 ± 6
HGF	8		105 ± 3	145 ± 9	136 ± 5
DHE + HGF	10	99 ± 3	100 ± 3	142 ± 11	138 ± 10

Nelson(6). The HGF preparation‡ which was used was stated to have a potency of 1 unit in 0.5 μg and less than 0.005 unit of insulin activity per mg. This preparation was named 'glucagon' by Staub *et al.*(7). Some preliminary studies were done using an amorphous insulin preparation (22 units per mg).‡ Injections were made intravenously. The dosages in mg per kg of the drugs injected were as follows: l-epinephrine bitartrate, 0.03; dihydroergotamine tartrate, 0.2; HGF, 1.0; $CoCl_2 \cdot 6H_2O$, 40. When DHE was used, it was injected 30 min. prior to the injection of the hyperglycemic agent.

Results. Differential blockade of HGF and epinephrine in vitro and in vivo. In order to determine whether DHE would interfere with the action of HGF upon liver slices, we have tested concentrations of HGF which produced much less than a maximum effect on glucose production. These conditions avoid the situation in which a large excess of HGF could effectively suppress the action of a reversible blocking agent. In Table I it may be seen that when a concentration of DHE was used such that the glycogenolytic effect of a sub-maximal concentration of epinephrine was partially or completely blockaded, the effects of an equi-active concentration of HGF were not diminished. A preliminary note mentioned these *in vitro* results(4). The first indication we obtained that HGF was not antagonized by DHE was in some student demonstrations on rabbits in which DHE was used to potentiate the hypoglycemic effects of insulin. When these rabbits showed evidence of hypoglycemic shock, epinephrine was of no benefit, whereas an amorphous insulin preparation (heated in alkaline solution to destroy insulin activity, but not HGF activity) appeared to produce a marked improvement in the condition of the insulin-treated animals.

A group of fasted rabbits were tested for their blood sugar response to the purified HGF preparation and to epinephrine, with or without prior treatment with DHE. The results are summarized in Table II. These results clearly demonstrated that the HGF-hyperglycemia was resistant to blockade by a dosage of DHE which completely suppressed epinephrine-hyperglycemia. These results indicated that a clear differentiation of the hyperglycemic effects of HGF and epinephrine could be made with the use of DHE. Since

‡ Generously supplied by Eli Lilly Research Laboratories, Indianapolis, through the courtesy of Dr. K. K. Chen.

TABLE III. Effect of DHE on Cobaltous Chloride Hyperglycemia.

	No. of tests	Avg blood sugar (mg %) ± S.E. Time before −30′	Time before 0′	Time after cobaltous chloride 30′	60′	120′
$CoCl_2$	7		95 ± 4	144 ± 12	164 ± 16	132 ± 9
DHE + $CoCl_2$	7	95 ± 5	95 ± 6	95 ± 6	98 ± 7	94 ± 8

the HGF-hyperglycemia was not influenced by DHE, the release of HGF from stimulated or damaged alpha-cells should result in a hyperglycemia in the DHE-treated rabbit.

Blockade of cobalt-hyperglycemia by DHE. An interesting application of the above information was suggested by a recent paper on the effects of cobaltous chloride on the blood sugar and alpha-cells of the pancreatic islets by Goldner, Volk, and Lazarus(1). The hyperglycemia produced by cobalt was not limited to the first administration, but occurred on repeated administration at a time when the alpha-cells were severely damaged. If this hyperglycemia were due to the direct or indirect stimulation of the adrenal medulla or to the stimulation of sympathetic nerves to the liver, then pretreatment with DHE should interfere with cobalt-hyperglycemia. On the other hand, a liberation of HGF even upon the first administration of cobalt should produce a hyperglycemia resistant to DHE. The results of administering cobalt with or without pretreatment with DHE on the blood sugar of fasted rabbits are summarized in Table III. These data include cross-over tests in which the rabbits which were pretreated with DHE upon the first administration of cobaltous chloride, were not given DHE before the next administration of cobalt and *vice versa*. It is quite evident from these data that cobalt-hyperglycemia is the result of a sympathetic discharge.

DHE on blood sugar of fasted, cobalt-treated rabbits. Following single or repeated treatment with cobaltous chloride sufficient to destroy the alpha-cells of the Islets of Langerhans(1) rabbits were fasted for 18-20 hours. After blood was obtained for control blood sugar level, DHE was injected. Blood samples were obtained over a period of 3 hours. The blood sugar was at the normal fasting level and did not change during this experimental period in spite of the supposed absence of HGF-producing cells and the blockade of sympathetic nervous activity on the liver.

Discussion. The selective blockade of epinephrine action without interference with the action of HGF implies a different mode of action of the two agents on the liver. Since both glycogenolytic agents produce their final effects by activating phosphorylase, the fact that selective blockade of one of them is possible would make their actions on the same site unlikely. It is evident that neither of the hyperglycemic drugs act directly upon phosphorylase, at least not in homogenized tissues (3). If several mechanisms for the reactivation of phosphorylase are available, it must be that epinephrine acts on one and HGF on another. A likely hypothesis is that epinephrine must first combine with its specific receptor on the cell in order for it to eventually exert its effects on glycogenolysis. This receptor is not used by HGF and thus only epinephrine action is blockaded by DHE.

There are few substances which increase the glucose production of liver slices, although several substances increase glycogenolysis without increasing glucose production. In the intact animal numerous chemicals and procedures increase blood sugar by an action on the liver. Since it is now established that even anoxia does not directly increase glucose production by liver slices(5), it would be well to determine whether agents reported to produce hyperglycemia are influencing blood sugar directly, or simply producing an effect through the mediation of the sympathetic nervous mechanism, or possibly by the liberation of HGF. To a limited extent this attitude has been accepted. Adrenalectomy is not sufficient to rule out an effect through the sympathetic nervous system. With the availability of a simple test such as the one used in this series of experiments, blood sugar effects may be analyzed quite readily.

It would appear that the technic of destruc-

tion of the alpha-cells with cobaltous chloride and the blockade of sympathetic effects with DHE (or related substances) should lend itself to a study of the mechanism of blood sugar control and the specific need for these hyperglycemic agents under varied conditions.

Summary. 1. DHE did not interfere with the glycogenolytic effect of HGF on rabbit liver slices or on hyperglycemia in intact rabbits. Comparable effects of epinephrine *in vitro* or *in vivo* were antagonized by DHE. 2. The hyperglycemia induced by cobaltous chloride, which destroys alpha-cells of the pancreatic islets, was prevented by DHE. This hyperglycemia is attributable to sympathetic nerve stimulation. No evidence for the release of HGF was obtained. 3. Blood sugar was maintained at a normal level in rabbits pretreated with cobalt, then fasted and injected with DHE to limit sympathetic action on the liver.

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Effect of Inositol Feeding on Inositol Phosphatides and Other Lipids of Human Blood Plasma.* (20654)

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Concerned by the role of atherosclerosis in producing hearing loss and labyrinthine dysfunction, it occurred to one of us that a lipotropic factor might be of some clinical value. A study of such possible effects of inositol was initated and has continued for the past several years. Biochemical as well as clinical methods were included. A method has been developed for the determination of lipid inositol in various tissues including blood plasma (1). Results of the determination of the effect of inositol ingestion on the concentration of plasma lipid inositol and several other plasma lipids are summarized in this paper.

Methods. All of the patients reported in this study showed varying degrees of high tone deafness and ranged from 34 to 68 years of age. One initial blood sample was obtained before oral inositol was started. The values for the various lipid components in these samples are in the normal range for the most part and the group could not be considered lipemic. The patients then all received 1 g of inositol† 3 times daily. Blood samples were obtained at varying intervals thereafter and averaged 2 samples per patient. The duration of inositol feeding in the patients reported ranged from 1 to 12 months. None of the patients reported any untoward symptoms or reactions from the inositol. All blood samples were obtained before breakfast between 9 and 9:30 a.m. 50 ml of blood was withdrawn into a centrifuge bottle containing 2 ml of Wintrobe's oxalate. The lipid extracts of the plasma were prepared, purified, and analyzed for total lipid phosphorus and inositol by methods reported previously(1,2). Total fatty acids were determined titrametrically by a modification of the method of Stoddard and Drury(3) on the purified extract.

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