Effects of Prolonged Glucagon Administration in the Cat and Dog

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It has been postulated that glucagon, the hyperglycemic factor of the pancreatic α-cells, plays an important role in the pathogenesis of diabetes mellitus in man.[1,2] This hypothesis suggests that an excessive secretion of glucagon in relation to insulin results in the diabetic state and is based on the finding of an increased α/β-cell ratio in the pancreatic islets of diabetic patients. One reason that the concept remains controversial is that efforts to induce permanent diabetes in the rat and the rabbit with glucagon have been unsuccessful.[3-7] However, these experiments may be considered inconclusive for the following reasons: The rat and the rabbit are relatively insensitive to the hyperglycemic action of glucagon;[8] they also respond poorly to some diabetogenic agents;[9] and the experimental conditions used were perhaps not ideal.[6,10] The purpose of this study was to determine the effects of prolonged glucagon administration by various routes (including the portal vein) in the two species (cat and dog) most sensitive to glucagon[8] and to certain known diabetogenic agents.[9]

METHODS

Experiments were carried out in six cats and seven dogs, all adult animals. Five different glucagon preparations were used: (1) No. 225-20-255* (20 per cent pure†); (2) No. 208-108B-234* (20 per cent pure); (3) No. 208-158B-197* (50 per cent pure); (4) crystalline glucagon‡ (100 per cent pure); and (5) crystalline Zn-glucagon§ (100 per cent pure). Only animals that received glucagon by continuous intraperitoneal infusion (except dog 559) were anesthetized (sodium pentobarbital); all others were conscious during glucagon administration. In the latter animals, glucagon was infused continuously by means of a gravity drip device regulated by an electronic valve, the details of which have been described elsewhere.[12] This apparatus permits continuous infusion in the conscious, unrestrained animal for prolonged periods (weeks to months). Heparin and penicillin were added to the infusion fluid to prevent thrombosis and infection. The animals' diet consisted of canned dog food (Pard) ad libitum and 20 gm. of sucrose two or three times daily. They were housed in metabolic cages for the collection of twenty-four-hour urine specimens. The doses of glucagon and the routes of administration are given in table 1. One cat (No. 4) and one dog (No. 658) were studied before and after partial pancreatectomy.

Blood sugar determinations were done by the Nelson modification of the Somogyi method.[13] Twenty-four-hour urine glucose was estimated by Benedict's quantitative method.[14]

RESULTS

In preliminary experiments, the hyperglycemic effect of glucagon (20 per cent pure) in various vehicles (water, peanut oil, cottonseed oil, and 15 per cent gelatin) given by three different routes (intramuscular, intravenous and intraperitoneal) was tested by means of a single injection or a five- to seven-hour continuous infusion. Of these methods only the aqueous preparations given intravenously or intraperitoneally by continuous infusion resulted in sustained hyperglycemia (220±60 mg. per cent) throughout the infusion period. All others gave only brief or insignificant blood sugar elevations. The Zn-glucagon suspension produced sustained but less marked hyperglycemia for three to five hours following a single intramuscular injection.

Both aqueous and Zn-glucagon preparations were, accordingly, administered for prolonged periods. Table 1

From the Radioisotope Service of the Veterans Administration Hospital and the Department of Medicine, University of Colorado School of Medicine, Denver, Colorado.

*Kindly supplied by Dr. O. K. Behrens, Eli Lilly and Company, Indianapolis.

†All purity figures are expressed in relation to the activity of the crystalline glucagon standard, which is designated 100 per cent pure.

‡Prepared from an amorphorus preparation supplied by Eli Lilly and Company, Indianapolis, by the method of Staub et al.[11]

§Prepared by Dr. A. Staub.

TABLE 1

Effect of prolonged glucagon administration in adult cats and dogs

Animal no.	Glucagon preparation (purity) per cent	Dose of glucagon preparation per 24 hours (mg.)	Method of administration	Route of administration	Duration of administration (days)	Effect on blood sugar	24 hr. urine sugar (gm.)	Remarks
Cat 11	20	0.5	Single inj. 3x/day	Intraperitoneal	8	Not measured	Slight incr.	Urine glucose returned to control levels when glucagon was stopped.
Cat 15	20	3.0	Single inj. 3x/day	Intraperitoneal	30	Not measured	Slight initial incr.	Urine glucose returned to control levels in spite of continued glucagon administration.
Cat 2	20	6.0	Single inj. 3x/day	Intraperitoneal	46	Hyperglycemia for 1-3 hrs. after each inj.: 145-196 mg.%	0-0.8	Blood and urine glucose values returned to control levels when glucagon was stopped.
Cat 3	20	3.0-5.0	Single inj. 2x/day	Intraperitoneal	108	Hyperglycemia for 1-3 hrs. after each inj.: 163-310 mg.%	0-2.0	Blood and urine glucose values returned to control levels when glucagon was stopped.
Cat 4	20	4.0-5.0	Cont. inf. for 5-7 hrs./day 5 days/wk.	Intraperitoneal	44 before pancreat. 13 after pancreat.	Hyperglycemia maintained during inf.: 155-252 mg.%	0.5-5.0	No change in response to glucagon after partial (50%) pancreatectomy. Blood and urine glucose values returned to control levels when glucagon was stopped.
Cat 5	20	4.0-5.0	Cont. inf. for 5-7 hrs./day 5 days/wk.	Intraperitoneal	13	Hyperglycemia maintained during inf.: 150-286 mg.%	0.5-3.0	Blood and urine glucose returned to control levels when glucagon was stopped.
Dog 378	20	5.0	Cont. inf. for 5-7 hrs./day 5 days/wk.	Intraperitoneal	5	Not measured	0	Urine glucose returned to control levels when glucagon was stopped.
Dog 478	50	5.0-15.0	Cont. 24-hr. inf.	Portal vein	27	Not measured	0-0.5	Urine glucose returned to control levels when glucagon was stopped.
Dog 503	50	11.0-18.0	Cont. 24-hr. inf.	Portal vein	9	Not measured	0.5-1.0	Urine glucose returned to control levels when glucagon was stopped.
Dog 505	50	10.0-15.0	Cont. 24-hr. inf.	Ext. jugular vein	9	Not measured	0.5-2.5	Urine glucose returned to control levels when glucagon was stopped.
Dog 559	50	10.0-15.0	Cont. 24-hr. inf.	Intraperitoneal	8	Not measured	0.5-2.0	Experiment terminated due to repeated clogging of catheter. Urine glucose changes only during glucagon administration.
Dog 658	100	0.4	Cont. 24-hr. inf.	Inferior vena cava	1st exp. 23 2nd exp. 21 (7 wks. later)	Not measured	0-0.5	75% of pancreas removed 2 mos. before first experiment. Urine glucose changes only during glucagon administration.
Dog 781	100 (Zn glucagon)	1.0	Single inj. 2x/day	Intramuscular	14	Not measured	0-0.5	Urine glucose changes only during glucagon administration.

summarizes the data of these experiments. It is noteworthy that although it was possible to maintain hyperglycemia and glycosuria in the cat for periods up to seven hours with constant intraperitoneal infusion of glucagon day after day, in no case did these persist after glucagon was stopped. In the dog, only minimal and transient glycosuria occurred despite continuous and prolonged glucagon administration. No toxic effects or weight loss occurred in any of the animals.

DISCUSSION

The experiments reported here suggest that large doses of glucagon are incapable of causing persistent hyperglycemia and glycosuria in the two species most sensitive to glucagon and certain known diabetogenic agents. Thus, they lend no support to the concept that glucagon is capable of producing permanent diabetes.

The following reports are consistent with the hypothesis that glucagon is involved in the pathogenesis of diabetes. Pituitary growth hormone, which is diabetogenic, has been reported to produce an increased α/β cell ratio,[15,16] and an increased α-cell activity.[16,17] The pancreatic venous blood (but not femoral vein blood) of animals treated with pituitary growth hormone has been found to contain a hyperglycemic substance(s).[18] Hypophysectomy, which ameliorates the diabetic state, has been reported to result in a decreased number of α-cells.[15,16] Glucagon has been reported to inhibit the action of insulin in the isolated rat diaphragm.[19,20] However, there are conflicting observations on each of the reports cited. Using more specific staining technics, Gomori[21] observed a normal α/β cell ratio in a high percentage of diabetics, and Seifert[22] reported an increased α/β ratio in many nondiabetics. The effects of hypophysectomy and pituitary growth hormone on pancreatic α-cells have not been confirmed by recent studies, which employed highly differential staining technics.[23,24] The assumption that the hyperglycemic substance found in the pancreatic vein blood of growth hormone treated animals is glucagon, is rendered questionable by a report that the hyperglycemic effect is eliminated by an adrenergic blocking agent.[25] Finally, there is newer evidence that glucagon does not inhibit glucose uptake in peripheral tissues,[26-28] but actually increases it in the normal and diabetic human.[29-31]

A number of laboratories have studied the effects of prolonged administration of glucagon in the rat and the rabbit. Root[5] administered large doses of glucagon daily for six months to rats intraperitoneally and to rabbits intravenously without producing diabetes or even sustained glycosuria. Ingle et al.[4] gave glucagon by continuous subcutaneous infusion for five days, and by continuous intrajugular infusion for eight days, to normal and partially depancreatized rats and noted only occasional mild glycosuria. Glucagon was found to produce a transient hyperglycemia and glycosuria in the force-fed normal rat, but not in the rat fed ad libitum, even when partially depancreatized.[3] Recently, Salter et al.,[6,7] have reported that glucagon administered intramuscularly caused marked hyperglycemia and glycosuria in force-fed rats. However, in no instance did permanent diabetes develop. The rats became increasingly ill after a few days and only two survived for as long as ten days. Root[5] observed no toxic effects in her rat experiments with doses of glucagon, comparable to those used by Salter et al., administered for six months. In the present studies no toxic effect or weight loss was observed when glucagon in doses up to 18 mg./day was administered for periods up to 108 days.

Salter et al.[6,7] have referred to glucagon as a "diabetogenic agent" and used the term "glucagon diabetes" to describe their findings. Diabetes has been defined as a syndrome characterized by persistent hyperglycemia and glycosuria associated with or resulting from impaired utilization of glucose. If this definition is valid, it seems to us that a distinction should be made between conditions which have been shown to produce this metabolic abnormality permanently (e.g. pancreatectomy, alloxan and pituitary growth hormone), those[9] which induce this state temporarily (e.g. adrenalin and adrenal steroids), and those[9] which produce transient hyperglycemia and glycosuria without impairment of glucose utilization (e.g. force-feeding). Most of the evidence to date appears to be in agreement that glucagon belongs in the third of these categories. Whether this category merits the designation "diabetes" is a debatable issue.

SUMMARY

Experiments in which large doses of glucagon were administered by various routes (including the portal vein) for prolonged periods to thirteen adult cats and dogs are reported. The hyperglycemia and glycosuria induced by glucagon were transient in all animals. The findings are in agreement with previous ones in species (rat and rabbit) which are less sensitive to glucagon and certain known diabetogenic agents than the cat and dog. They lend no support to the hypothesis that glucagon is involved in the pathogenesis of diabetes mellitus.

SUMMARIO IN INTERLINGUA

Effectos De Prolongate Administrationes De Glucagon In Cattos E Canes

Es reportate experimentos in que grande doses de glucagon esseva administrate per varie vias (incluse le vena portal) e durante prolongate periodos de tempore a decetres adulte cattos e canes. Le hyperglycemia e glycosuria inducite per glucagon esseva transiente in omne le animales. Le constatationes es identic con illos previemente facite in species—i.e. rattos e conilios—que es minus sensibile a glucagon e certe cognoscite agentes diabetogene. Esseva trovate nulle supporto pro le hypothese que glucagon participa in le pathogenese de diabete mellite.

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