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GLYCOSTATIC EFFECT OF A DIABETOGENIC NON-GROWTH-PROMOTING PITUITARY POLYPEPTIDE*

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ABSTRACT

1. Treatment with 2 mg. of a non-growth-promoting bovine diabetogenic peptide or growth hormone in two divided doses, one at the beginning and one 7–9 hours later, to hypophysectomized rats during a 24-hour fast maintained their muscle glycogen at a normal level.

2. Administration of bovine serum albumin or saline was accompanied by a sharp decrease of muscle glycogen.

3. The minimum dose of highly purified bovine diabetogenic peptide which is capable of maintaining muscle glycogen at normal levels was found to be 0·2–0·3 mg. in 135–150-g. rats.

4. Diabetogenic peptide maintains muscle glycogen in fasted hypophysectomized-adrenalectomized rats.

5. Diabetogenic peptide does not promote growth as measured by the tibia test.

In recent work from this laboratory (Louis, Conn, and Minick, 1963; Louis and Conn, 1969) isolation of an insulin antagonist from the urine of patients with lipoatrophic diabetes and from diabetic patients with proteinuria has been reported. It was also demonstrated that a similar substance can be isolated from bovine, ovine, and porcine adenohypophyses (Louis, Conn, and Minick, 1966; Louis and Conn, 1968). The substance is a polypeptide. When administered either to dog or man it induces resistance to exogenous insulin and reduces tolerance for glucose. The present report describes a glycostatic effect of this peptide, similar in nature to that exerted by growth hormone.

When food is withheld from hypophysectomized rats, the carbohydrate stores of the body are quickly depleted. During a 24-hour fasting period, liver glycogen and blood-glucose begin to fall within a few hours, and later muscle (gastrocnemius) glycogen declines to levels well below those seen in normal, fasting rats. During a 24-hour fast of hypophysectomized rats, muscle glycogen can be maintained at normal values by treatment with alkaline anterior pituitary extracts. This phenomenon has been called the ' glycostatic effect '. This area of research has been carefully reviewed by Russell (1954). Russell and Wilhelmi (1950) have also demonstrated that purified growth hormone exerts the ' glycostatic effect '.

The present work indicates that another diabetogenic peptide, distinct from growth hormone, also exerts the glycostatic effect.

MATERIALS AND METHODS

Hypophysectomized and normal male rats of the Sprague-Dawley strain have been used for studies of the glycostatic effect and were obtained from Simonsen Labs Inc., Gilroy, California. Hormone Assay Laboratory, Chicago, provided the hypophysectomized male rats used for tibia tests. The hypophysectomized rats were obtained 5 days postoperatively and were maintained on a rat pellet diet. Complete atrophy of the gonads and accessory organs was accepted as indicating complete removal of the anterior lobe. Studies

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were carried out between 2 weeks and 2 months after hypophysectomy. No differences were observed in the responses of animals studied soon (2 weeks) or late (2 months) after operation. Adrenalectomies were done through a bilateral lumbar approach and the region of the adrenal was inspected for accessory cortical tissue or cortical remnants at autopsy. Adrenalectomized rats were provided with a 1 per cent NaCl solution as drinking water *ad libitum*. Hypophysectomized-adrenalectomized rats were studied and sacrificed within 72 hours after completion of the adrenalectomy.

The diabetogenic material from bovine adenohypophysis was isolated by a procedure described previously (Louis and others, 1966). The purified diabetogenic polypeptide preparation was obtained by further purification of the material on Sephadex G-100 (Tutwiler and Louis, 1970). The diabetogenic activity of this material was demonstrated in dogs. The peptide was prepared for injection in the same way as that previously described (Louis and others, 1963).

Bovine growth hormone (NIH-GH-B8) was dissolved in 0·9 per cent NaCl, with the addition of a small amount of 0·1 *N* NaOH. The bovine serum albumin employed was dissolved in 0·9 per cent NaCl.

The experimental animals were given saline, bovine serum albumin, bovine growth hormone, or our diabetogenic polypeptide preparations intraperitoneally in 2 equal doses, 1 at the beginning of the 24-hour fasting period and the second 7–9 hours later. The volume of solution injected per dose was 0·5–1·0 ml.

At the end of the 24-hour experimental period, the animals were sacrificed by decapitation. The whole livers were removed and 2 separate pieces (approximately 1 g.) were weighed and immediately frozen. The 2 gastrocnemii were removed, weighed, and frozen. The liver sections and gastrocnemii were then placed in 15-ml. centrifuge tubes containing 30 per cent KOH. The isolation and hydrolysis of glycogen were carried out by a slight modification of the procedure described by Zarrow, Yochim, and McCarthy (1964). Glucose was measured by the Somogyi-Nelson procedure (Somogyi, 1952). Muscle and liver glycogen values for each animal are averages of 2 separate determinations.

The rats used for tibia tests weighed 60–80 g. and had been hypophysectomized at 20–30 days of age. The tibia tests were performed by the procedure of Greenspan, Li, Simpson, and Evans (1949).

Table I.—Glycogen and Blood-glucose Values of Fasted and Non-fasted, Hypophysectomized, and Intact Rats

Treatment of Rats	Number of Rats	Age (days)	Weight (g.)	Muscle Glycogen (mg. per 100 g.)	Liver Glycogen (mg. per 100 g.)	Blood-glucose Range (mg. per 100 ml.)
A. Non-fasted						
1. Hypophysectomized	10	51–72	130–145	330±10	2287±127	115–121
2. Intact	9	35–60	145–265	303±10	4890±122	120–133
B. Fasted 24 hours						
1. Hypophysectomized	9	47–56	120–140	49±1	13±1	44–54
2. Hypophysectomized	5	54–57	130–135	65±2	14±1	36–46
3. Intact	10	33–39	113–161	205±6	15±1	76–92

Data expressed as average±standard error.

RESULTS

The values obtained for muscle and liver glycogen and for blood-glucose of normal and hypophysectomized rats are summarized in *Table I.* Hypophysectomized rats fasted for 24 hours and given saline injections showed an abnormal decrease in blood glucose and muscle glycogen when compared with fasted intact rats. The liver glycogen of hypophysectomized rats was only slightly less than that of intact rats fasted 24 hours. These results are similar to those reported by Russell and Bennett (1937).

The effects of treating hypophysectomized rats with bovine serum albumin, bovine growth hormone, and our diabetogenic polypeptide are summarized in *Table II.* It is apparent that 2 mg. of bovine growth hormone or 2 mg. of bovine diabetogenic polypeptide were sufficient to maintain their

muscle glycogen at normal levels. In an attempt to show that the effects of these two hormones were not non-specific effects of njected protein, bovine serum albumin and an inactivated form of our peptide (precipitated at *p*H 1·9–2·0) were used as controls. It is clear that these compounds are incapable of maintaining muscle glycogen in hypophysectomized rats during a 24-hour fast.

Table III shows the minimum effective dose (total amount given during the fast that can prevent depletion of liver and muscle glycogen) of the purified diabetogenic polypeptide in fasted hypophysectomized rats. The minimum effective dose under the conditions described above is 0·2–0·3 mg. per rat. This is similar to the minimum effective dose of purified growth hormone as shown

Table II.—Glycogen and Blood-glucose Values in Fasted Hypophysectomized Rats treated with Bovine Serum Albumin and Adenohypophysial Polypeptides

Treatment	Number of Rats	Age (days)	Weight (g.)	Muscle Glycogen (mg. per 100 g.)	Liver Glycogen (mg. per 100 g.)	Blood-glucose Range (mg. per 100 ml.)
2 mg. bovine serum albumin	6	43–53	130–142	65±2 $P<1$*	11·7±1 $P<0·3$	48–56
2 mg. bovine growth hormone	9	43–72	130–143	217±3 $P<0·001$	16±0·4 $P<0·001$	45–52
2 mg. bovine adenohypophysial diabetogenic polypeptide	10	50–60	133–145	220±2 $P<0·001$	16±0·2 $P<0·001$	40–50
2 mg. bovine adenohypophysial inactive material (precipitated at *p*H 1·9–2·0)	5	53–60	130–137	84±2 $P<0·001$	13±1 $P<·9$	38–50

* Student's *t*-test values computed in comparison with hypophysectomized rats fasted 24 hours.

Table III.—Minimum Effective Dose of Diabetogenic Polypeptide on Muscle and Liver Glycogen of Fasting Hypophysectomized Rats

Experiment	Number of Rats	Age (days)	Weight (g.)	Purified Peptide (mg.)	Muscle Glycogen (mg. per 100 g.)	Liver Glycogen (mg. per 100 g.)
Control rats	9	47–56	120–140	0	49±1	13±1
Treated rats	3	53–59	130–135	1·0	209±10 $P<0·6$*	14±1 $P<0·6$
	4	50–61	139–150	0·5	211±2 $P<0·1$	16±1 $P<0·8$
	5	47–52	134–149	0·3	208±3 $P<0·5$	17±1 $P<0·02$
	5	48–62	130–145	0·2	172±4 $P<0·001$	16±1 $P<0·2$

* Student's *t*-test values computed in comparison with fasting intact rats. Muscle glycogen 205±6 mg. per 100 g.; liver glycogen 15±1 mg. per 100 g.

by Russell and Wilhelmi (1950). These workers found the dose to be 0·1–0·2 mg. per 100 g. *of rat body-weight.*

To determine whether the diabetogenic polypeptide could maintain muscle and liver glycogen levels of fasted hypophysectomized rats in the absence of the adrenal glands, 2 mg. of the polypeptide were administered to hypophysectomized-adrenalectomized rats. The results, *Table IV*, indicate the glycostatic effect of our peptide in the doubly operated preparation.

normal levels of muscle glycogen and blood-sugar, and (2) fasting hypophysectomized rats exhibit tremendous depletion of these carbohydrate stores as compared with fasting intact rats.

The loss of muscle glycogen in the fasted hypophysectomized rat can be prevented by administration of pituitary extracts (Russell, 1954) or highly purified growth hormone (Russell and Wilhelmi, 1950). Our data with bovine growth hormone (*Table III*) confirm those findings. In addition, however, they

Table IV.—Glycogen and Blood-glucose Values in Fasted Hypophysectomized-Adrenalectomized Rats treated with Bovine Adenohypophysial Diabetogenic Polypeptide

Peptide (mg.)	Number of	Age (days)	Weight	Muscle Glycogen (mg. per 100 g.)	Liver Glycogen (mg. per 100 g.)	Blood-glucose Range
2	6	48–55	135–150	236 ± 10 $P<0.001$*	16.9 ± 1 $P<0.05$*	40–60
0	4	49–50	138–145	53 ± 3 $P<0.5$†	13 ± 1 $P<0.9$†	38–46

* Student's *t*-test values computed in comparison with hypophysectomized-adrenalectomized rats, fasted 24 hours.

† Student's *t*-test values computed in comparison with hypophysectomized rats, fasted 24 hours.

Table V.—Assay for Growth-promoting Activity by the Tibia Test (10)

Treatment	Number of Rats	Width Uncalcified Cartilage (μ)
Controls	7	175 ± 5
40 μg. growth hormone	4	256 ± 4 $P<0.001$
1000 μg. diabetogenic polypeptide	6	174 ± 3 $P<.8$

Table V demonstrates that 1000 μg. of our diabetogenic polypeptide are not growth-promoting when measured according to the tibia test of Greenspan and others (1949).

DISCUSSION

The control data (*Table I*) of the present experiments confirm previous reports that (1) well-fed hypophysectomized rats maintain

demonstrate that our diabetogenic polypeptide from bovine adenohypophysis is potent with respect to this 'glycostatic effect'. Our results indicate, too, that adrenal cortical function is not essential for the maintenance of muscle glycogen by our diabetogenic polypeptide, a phenomenon previously documented for pituitary extracts (Bennett, 1938; Bennett and Perkins, 1945).

Because of the similarity in the 'glycostatic effects' of bovine growth hormone and our diabetogenic polypeptide, we have compared the growth-promoting activity of these two substances as measured in the tibia test of Greenspan and others (1949), *Table V*. It is clear that the diabetogenic polypeptide, even when given in very large amounts, has no activity in this respect.*

* Dr. Don S. Shalch, School of Medicine and Dentistry of the University of Rochester, showed that our crude peptide has about one-thousandth the affinity for antigrowth hormone antibody as bovine growth hormone.

It is also of interest that bovine growth hormone does not (Lewis, Klein, and Wilkins, 1950) while our diabetogenic polypeptide does (Louis and others, 1966) diminish carbohydrate tolerance in humans. Bovine growth hormone can be altered by chemical manipulation after which it may affect carbohydrate tolerance in man (Sonenberg, Free, Dellacha, Bonadonna, Haymowitz, and Nadler, 1965) but the altered molecule retains growth-promoting activity. The possibility that the diabetogenic peptide is an altered moiety of growth hormone cannot be excluded by our present data. However, it is unlikely that the growth hormone molecule could be disrupted by the mild treatment employed in our isolation procedure.

CONCLUSION

The diabetogenic polypeptide reported by our laboratory to be present in the urine of patients with lipoatrophic diabetes, diabetic patients with proteinuria, and, subsequently, to be present in bovine, ovine, and porcine adenohypophysis, has now been demonstrated to exert a glycostatic effect in fasting hypophysectomized rats. While pharmacologically similar to growth hormone in several respects, the diabetogenic peptide possesses no growth-promoting activity and differs from growth hormone on a physicochemical basis. Since it is unlikely that this peptide represents an altered growth hormone molecule, it is possible that this polypeptide, rather than growth hormone, may be implicated in the pathogenesis of diabetes mellitus and for its complications in man.

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