SHORT COMMUNICATION

INFLUENCE OF BOVINE DIABETOGENIC PEPTIDE, GROWTH HORMONE, AND PROLACTIN ON TISSUE GLYCOGEN, LIVER FAT, AND LIVER WATER OF FASTING RATS

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ABSTRACT

1. Bovine diabetogenic peptide, growth hormone, and prolactin were compared with respect to their effects on tissue glycogen, liver fat, and liver water in fasting rats.
2. Bovine diabetogenic peptide increases liver size, liver glycogen, liver water, and total liver lipids.
3. Bovine diabetogenic peptide, in contrast to growth hormone and prolactin, does not increase muscle glycogen levels.

In recent work from this laboratory (Louis, Conn, and Minick, 1963; Louis and Conn, 1969a, b), the isolation of an insulin antagonist from the urine of patients with lipoatrophic diabetes and from proteinuric diabetic patients without lipoatrophy has been reported. It was also demonstrated that a similar substance can be isolated from bovine, ovine, and porcine adenohypophysis (Louis, Conn, and Minick, 1966; Louis and Conn, 1968). The substance is a polypeptide which, when administered to either dog or man, exhibits diabetogenic and anti-insulin effects. The bovine substance is not growth-promoting and maintains muscle glycogen in fasting hypophysectomized rats (Tutwiler and Louis, 1971). The diabetogenic and glycostatic effects are similar to those previously elucidated for the growth hormone (BGH) and prolactin. The present report represents an attempt to delinate further biological differences between bovine diabetogenic peptide, growth hormone, and prolactin.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 38–40 days of age, were fasted for 24 hours; on the second day of fasting, 4·0 mg. of bovine diabetogenic peptide, growth hormone, or prolactin in 3 divided doses at 2-hour intervals were administered intraperitoneally. Control animals were given either saline or 4 mg. bovine serum albumin (BSA) in a similar manner.

The bovine diabetogenic peptide was prepared and dissolved by a procedure prescribed previously (Louis and others, 1966). BGH (NIH-GH-B_8) was dissolved in 0·9 per cent NaCl with the addition of a small amount of 0·1 *N* NaOH. Bovine prolactin (NIH-P-B1) and BSA were dissolved on 0·9 per cent NaCl.

Two hours after the last injection the animals were sacrificed by decapitation. The whole liver, heart, diaphragm, and gastrocnemius muscle were removed, weighed, and frozen. Liver tissue from each rat was divided into 3 parts, weighed, and used respectively for total lipid, glycogen, and water determination. The liver tissue for water determination was placed in a tared glass tube and dried to constant weight *in vacuo*. The difference in weight before and after drying was taken as the water content. The isolation and hydrolysis of glycogen from the various tissues were carried out by the procedure of Zarrow, Yockin, and

McCarthy (1964). Glucose determinations on these hydrolysates were measured by the Somoygi-Nelson procedure (Somogyi, 1952). Liver total lipids were determined by a slight modification of the method of Folch, Lees, and Stanley (1957).

RESULTS AND DISCUSSION

The changes in liver composition of fasted rats after treatment with bovine diabetogenic peptide, growth hormone, and prolactin are summarized in *Table I*. It is evident that the livers of diabetogenic-, growth-, or prolactin-treated rats have a higher wet weight and glycogen content than the control rats. Water content of liver tissue is not significantly altered by BGH or prolactin treatment; however, liver water content is significantly increased in the diabetogenic peptide treated rats. A somewhat higher fat content is also seen in diabetogenic peptide or BGH treated rats. The earlier experiments of Best and Campbell (1938) established the fact that certain anterior pituitary extracts produce a rapid increase in size and an intense fatty infiltration of the liver in fasting rats. Li, Simpson, and Evans (1949) observed an increase in fat content of the liver when growth hormone was administered to fasting normal and hypophysectomized rats. The present experiments reconfirm the ability of BGH to increase liver fat in fasting normal rats. The

Table I.—EFFECT OF BOVINE DIABETOGENIC PEPTIDE, GROWTH HORMONE, AND PROLACTIN ON THE LIVER COMPOSITION OF FASTED RATS*

HORMONE INJECTED	NO. OF RATS	BODY-WEIGHT (g.)	LIVER SIZE (g. per 100 g. body-weight)	LIVER COMPOSITION		
				Water (per cent)	Fat (mg. per g. dry weight)	Glycogen (mg. per 100 g. wet weight)
Diabetogenic peptide	12	190–219	3·10 ± 0·05 $P<0·001$	72·6 ± 0·5 $P<0·001$	165 ± 3 $P<0·001$	342 ± 38 $P<0·001$
Growth hormone	9	174–205	3·34 ± 0·05 $P<0·001$	70·65 ± 0·21 $P<0·05$	159 ± 4 $P<0·005$	281 ± 5 $P<0·001$
Prolactin	8	161–203	3·0 ± 0·04 $P<0·05$	69·7 ± 0·4 $P<0·9$	142 ± 2 $P<0·7$	386 ± 59 $P<0·001$
Saline	15	160–205	2·80 ± 0·57	70·4 ± 0·1 $0·05<P<0·1$	149 ± 2	78 ± 7
BSA	10	170–230	2·82 ± 0·05 $0·8<P<0·9$	69·7 ± 0·4	144 ± 3 $P>0·9$	86 ± 13 $0·5<P<0·6$

* Results expressed as mean ± S.E. P computed from Student's t-test analysis.

Table II.—EFFECT OF BOVINE DIABETOGENIC PEPTIDE, GROWTH HORMONE, AND PROLACTIN ON TISSUE GLYCOGEN LEVELS OF FASTED RATS

HORMONE INJECTED	NO. OF RATS	GLYCOGEN CONTENT (mg. per 100 g.)		
		Gastrocnemius	Cardiac Muscle	Diaphragm
Diabetogenic peptide	12	165 ± 9 $0·6<P<0·7$	225 ± 15 $0·7<P<0·8$	115 ± 10 $P<0·9$
Growth hormone	9	258 ± 14 $P<0·001$	373 ± 19 $P<0·001$	171 ± 7 $P<0·001$
Prolactin	8	217 ± 12 $P<0·02$	322 ± 22 $P<0·02$	160 ± 7 $P<0·001$
Saline	15	187 ± 7 $0·2<P<0·3$	205 ± 10 $0·3<P<0·4$	108 ± 5 $0·2<P<0·3$
BSA	10	171 ± 11	235 ± 9	116 ± 7

present study shows that the diabetogenic peptide from bovine adenohypophysis also possesses this property. Since under these circumstances the accumulation of lipid in the liver is an indirect measure of fatty acid mobilization from adipose tissue, it is likely that the diabetogenic peptide possesses a lipid-mobilizing effect similar to BGH. This possibility is now being tested.

Muscle glycogen levels following the various procedures are shown in *Table II*. BGH- and prolactin-treated animals have higher muscle glycogen than the controls. The diabetogenic peptide exhibits no effect on muscle glycogen. Russell (1955) described the growth-hormone-stimulated increase in cardiac glycogen in fasting intact rats as a growth property. The two known growth-promoting peptides, BGH and prolactin, have here again been shown to increase cardiac glycogen while the non-growth-promoting diabetogenic peptide does not exert this effect.

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Key Word Index: Bovine diabetogenic peptide, growth hormone and prolactin, comparative effects *in vivo*, glycogenic, liver fat increase.