Induction of Hyperinsulinemia and Hyperglycemia in Dogs by Administration of Diabetogenic Bovine Pituitary Peptide

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A peptide exhibiting diabetogenic and anti-insulin properties isolated from bovine adenohypophysis was administered subcutaneously in single doses to four dogs for 2 to 3 consecutive days. Ten hours after each injection, the animals were given an oral glucose load and a series of blood samples were obtained for glucose and insulin determinations. In one dog, the procedure was repeated 13 days after the first one. In another animal, the same procedure was carried out three times. Glucose tolerance was clearly impaired and serum insulin rose excessively in three of the four animals. In the fourth animal the response was observed but only after the third injection. Thus, insulin resistance appears to be a major mechanism by which this peptide induces loss of carbohydrate tolerance. (Metabolism 20: No. 3, March, 326-330, 1971)

PREVIOUS PUBLICATIONS from this laboratory have demonstrated the presence of a diabetogenic peptide in the urine of patients with lipoatrophic diabetes[1] and of proteinuric diabetic patients without lipoatrophy.[2] The substance is not present in the urine of proteinuric patients without diabetes, diabetics without proteinuria and healthy people.[2] A similar diabetogenic peptide has also been isolated from the adenohypophyses of beef,[3] hog and sheep.[4] The peptide from all of these sources produces hyperglycemia and induces antagonism to the action of exogenous insulin when administered to dogs or man. The mechanism by which the peptide acts has not been known but it was inferred from the above mentioned findings that resistance to endogenous insulin was probably a major factor.

In an attempt to study this possibility we have administered subcutaneously in dogs the peptide obtained from bovine adenohypophysis and have measured the serum insulin and blood glucose responses to glucose administered by gastric tube. The results indicate that resistance to endogenous insulin is a major factor in the mechanism by which the peptide induces hyperglycemia.

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Table 1

Dog K♂ 14.1 Kg Body Wt.

Effect of Bovine PI Upon Glucose Tolerance and Serum Insulin Level

	F	1/2	1	1 1/2	2	2 1/2	3
Glucose mg%							
Control (1)	83	107	89	80	81	80	85
Control (2)	84	126	78	81	88	79	78
10 hrs. after 1st inj. 3 mg/kg	87	201	165	101	89	92	72
10 hrs. after 2nd inj. 4 mg/kg	75	178	265	150	118	84	84
10 hrs. after 3rd inj. 5 mg/kg	102	215	226	145	115	106	106
34 hrs. after 3rd inj.	99	138	124	97	96	93	92
Insulin uU/ml							
Control (1)	5	6	4	4	6	15	9
Control (2)	12	73	32	22	12	10	11
10 hrs. after 1st inj. 3 mg/kg	18	112	47	48	15	15	34
10 hrs. after 2nd inj. 4 mg/kg	69	173	180	94	68	68	49
10 hrs. after 3rd inj. 5 mg/kg	45	229	126	78	43	43	38
34 hrs. after 3rd inj.	38	164	28	26	26	26	20

Table 2

Dog O♀ 11.3 Kg Body Wt.

Effect of Bovine PI Upon Glucose Tolerance and Serum Insulin Level

	F	1/2	1	1 1/2	2	2 1/2	3
Glucose mg%							
Control (1)	83	111	99	78	75	79	80
Control (2)	78	110	108	85	72	73	79
10 hrs. after 1st inj. 3 mg/kg	82	107	132	106	82	76	79
10 hrs. after 2nd inj. 4 mg/kg	74	115	120	118	87	81	73
10 hrs. after 3rd inj. 5 mg/kg	92	168	167	182	112	85	84
34 hrs. after 3rd inj.	90	119	100	119	94	79	83
Insulin uU/ml							
Control (1)	10	49	34	6	9	7	4
Control (2)	16	59	88	21	14	14	21
10 hrs. after 1st inj. 3 mg/kg	18	35	57	38	17	16	17
10 hrs. after 2nd inj. 4 mg/kg	19	53	71	66	41	19	24
10 hrs. after 3rd inj. 5 mg/kg	24	138	107	132	69	30	21
34 hrs. after 3rd inj.	48	116	104	84	28	16	32

Materials and Methods

Isolation of a diabetogenic peptide, designated as PI, from bovine adenohypophysis has been described.[3] The isolated substance is prepared for injection by solution in dilute hydrochloric acid (0.05–0.1 *N*) and neutralization with 0.05 *N* NaOH to pH 7.0–7.4. The solution has been administered subcutaneously as a single dose.

Four trained dogs, one female and three males, were studied. They were maintained between tests on a diet of Friskies Mix dog food and one can of Pard. For 3 days prior to and throughout the testing periods, the daily diet consisted of 454 Gm. of Pard, 100 Gm. Friskies and 60 Gm. of sucrose. No food was eaten after 10:00 p.m. Oral (gastric intubation) glucose tolerance tests were begun between 8:00 and 9:00 a.m. The experimental procedure consisted of two control glucose tolerance tests done on days 1 and 2; a subcutaneous injection of the peptide 10 hours before the third glucose tolerance test which was done on day 3; administration of a single dose of the peptide daily for two more days with glucose tolerance tests being done on days 4 and 5; and a final glucose tolerance test carried out 34 hours after the last injection of peptide (day 6). This series of tests is designated as "Test Period I." Postpeptide glucose tolerance tests were carried out 13 and 14 days after the last administration of peptide. Following an interval of at least 13 days, a second similar testing period, "Test Period II," was begun. In one dog, "Test Period III" was also performed. Blood glucose was determined by the Somogyi-Nelson Procedure.[5] Serum insulin was measured by the radioimmunoassay technique of Yalow and Berson.[6] Campbell et al. had shown that values obtained by the immunoassay procedure were actually representative of insulin concentrations in dog serum.[7]

Results

Blood glucose and serum insulin values for Dog K are presented in Table 1. Both insulin and glucose levels are greatly elevated by injection of this compound. Thirteen and fourteen days after peptide administration, the values had returned to normal. Dog O, Table 2, showed no significant change until the third day of peptide injection. There then occurred a mild rise of serum insulin and a marked impairment of glucose tolerance.

The results of Dog T are depicted in Tables 3 and 4. It is apparent that glucose tolerance was greatly impaired and that serum insulin levels rose sharply.

Table 3

Dog T♂ 13 Kg Body Wt.

(Test Period I)

Effect of Bovine PI Upon Glucose Tolerance and Serum Insulin Level

	F	1/2	1	1 1/2	2	2 1/2	3
Glucose mg%							
Control (1) (bovine albumin 4 mg/kg)	75	-	72	61	64	67	67
Control (2)	93	131	113	78	76	87	84
10 hrs. after 1st inj. 4 mg/kg	67	137	220	283	143	83	69
10 hrs. after 2nd inj. 5 mg/kg	89	175	236	230	136	99	93
10 hrs. after 3rd inj. 6 mg/kg	77	132	147	125	132	106	77
34 hrs. after 3rd inj.	72	165	92	67	77	65	80
Insulin uU/ml							
Control (1)	8	-	13	9	6	8	9
Control (2)	7	98	73	10	2	6	4
10 hrs. after 1st inj. 4 mg/kg	30	72	94	109	107	14	13
10 hrs. after 2nd inj. 5 mg/kg	43	175	154	208	89	27	54
10 hrs. after 3rd inj. 6 mg/kg	26	263	230	147	183	32	19
34 hrs. after 3rd inj.	14	137	34	27	27	44	36

Table 4

Dog T♂ 13 Kg Body Wt.

(Test Period II)

Effect of Bovine PI Upon Glucose Tolerance and Serum Insulin Level

	F	1/2	1	1 1/2	2	2 1/2	3
Glucose mg%							
Control (1) (bovine albumin 4 mg/kg	66	90	94	96	66	54	59
Control (2)	71	90	103	68	61	66	64
10 hrs. after 1st inj. 4 mg/kg	90	156	163	150	88	90	92
10 hrs. after 2nd inj. 5 mg/kg	100	175	203	191	111	86	76
10 hrs. after 3rd inj. 6 mg/kg	99	178	207	230	136	109	86
34 hrs. after 3rd inj.	68	124	165	143	84	70	75
Insulin uU/ml							
Control (1)	9	96	108	116	15	8	8
Control (2)	14	116	123	25	10	14	7
10 hrs. after 1st inj. 4 mg/kg	169	414	302	378	68	113	88
10 hrs. after 2nd inj. 5 mg/kg	214	454	544	686	146	235	147
10 hrs. after 3rd inj. 6 mg/kg	243	550	688	596	676	606	316
34 hrs. after 3rd inj.	87	249	254	354	66	22	30

In both test periods, there occurred an important rise of the *fasting* serum insulin level as well. By 13 and 14 days after the last injection of peptide, blood glucose and insulin levels had returned to normal. The results on Dog L are shown in Tables 5 and 6. The responses of this animal are very similar to those of Dog T, except that they are more intense. Again, the *fasting* serum insulin value was greatly elevated. However, in "Test Period III," glucose tolerance was normal and serum insulin was only mildly elevated. The reason for this finding is unclear at present. Perhaps a sufficient antibody titer had been produced to overcome the activity of the administered material. Studies in this area are in

Table 5

Dog L♂ 15.9 Kg Body Wt.

(Test Period I)

Effect of Bovine PI Upon Glucose Tolerance and Serum Insulin Level

	F	1/2	1	1 1/2	2	2 1/2	3
Glucose mg%							
Control (1) (bovine albumin 4 mg/kg)	77	100	100	102	71	81	79
Control (2)	76	147	127	84	74	79	80
10 hrs. after 1st inj. 4 mg/kg	70	166	192	212	134	93	91
10 hrs. after 2nd inj. 4 mg/kg	93	154	254	288	228	159	141
34 hrs. after 2nd inj.	78	146	205	229	161	121	100
58 hrs. after 2nd inj.	71	139	178	189	115	65	72
Insulin uU/ml							
Control (1)	12	136	104	136	9	9	11
Control (2)	16	181	218	98	9	25	10
10 hrs. after 1st inj. 4 mg/kg	42	288	310	352	259	100	43
10 hrs. after 2nd inj. 4 mg/kg	159	430	381	429	449	334	284
34 hrs. after 2nd inj.	58	342	376	392	407	398	101
58 hrs. after 2nd inj.	46	354	368	352	176	44	32

Table 6

Dog L♂ 15.9 Kg Body Wt.

(Test Period II)

Effect of Bovine PI Upon Glucose Tolerance and Serum Insulin Level

	F	1/2	1	1 1/2	2	2 1/2	3
Glucose mg%							
Control (1) (bovine albumin 4 mg/kg)	79	119	101	89	78	77	77
Control (2)	76	128	106	102	64	71	70
10 hrs. after 1st inj. 4 mg/kg	77	107	107	109	74	73	74
10 hrs. after 2nd inj. 5 mg/kg	91	137	171	189	155	95	87
10 hrs. after 3rd inj. 6 mg/kg	109	169	255	291	255	211	173
34 hrs. after 3rd inj.	103	155	306	274	237	170	142
Insulin uU/ml							
Control (1)	23	135	143	72	9	9	13
Control (2)	19	130	160	81	10	6	17
10 hrs. after 1st inj. 4 mg/kg	41	215	257	142	19	8	7
10 hrs. after 2nd inj. 5 mg/kg	57	347	574	303	245	45	56
10 hrs. after 3rd inj. 6 mg/kg	224	514	651	521	566	508	552
34 hrs. after 3rd inj.	125	370	555	532	472	386	266

progress. It should be noted that for Dogs T and L, the doses of peptide administered were somewhat larger than those given to Dogs K and O.

COMMENT

The present study demonstrates that administration of our bovine diabetogenic peptide to dogs results in hyperinsulinemia together with impairment of glucose tolerance. It has been shown that bovine growth hormone is also capable of inducing simultaneously hyperinsulinemia and hyperglycemia in dogs.[7-9] Because growth hormone and our peptide have similar diabetogenic properties, we have compared the growth promoting activity of these two peptides by means of the tibia test of Greenspan et al.[10] It was found that our diabetogenic peptide has no significant growth promoting activity.[11] It has been shown that bovine growth hormone can be degraded by trypsin or chymotrypsin after which it may be capable of inducing carbohydrate intolerance in man.[12,13] The possibility that our diabetogenic polypeptide is a part of the growth hormone molecule cannot be excluded completely but it is an unlikely one, since no diabetogenic peptide at pH 4.1 was obtained when bovine growth hormone (NIH-GH-B8) itself was subjected to our procedure of isolation. Previous results from this laboratory have shown that our substance has no ACTH effects in man.[3] No significant prolactin activity as measured by the Reece-Turner intradermal pigeon crop technique could be demonstrated.[4] Thus, it seems reasonable to conclude that our substance is different from other known pituitary hormones.

The possibility exists that this substance may induce beta cell release of abnormal quantities of an immunodetectable moeity of insulin with low biological activity, such as proinsulin. We have not yet made these measurements but such a mechanism could not explain the entire phenomenon, since we have already found[1-4] decreased sensitivity to intravenously administered exogenous insulin in dogs and men treated with this compound.

It is premature to speculate upon the possible physiological significance of this substance but it is important that we have recently isolated it from pituitary glands of man.[15]

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