Insulin and Insulinlike Activity in Extracts of Tumors Associated with Hypoglycemia

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SUMMARY

Insulin assays were performed on tissue extracts from ten nonpancreatic tumors and five islet-cell adenomas, all of which were associated with hypoglycemia. Acid alcohol and normal saline extractions were made from tumor tissue as well as from normal tissue. The insulin content of extracts was determined both biologically and immunologically, utilizing the rat epididymal fat pad to measure insulinlike activity (ILA) and the double antibody radioimmunoassay to estimate insulin (IRI).

All five islet-cell adenomas were found to have significant concentrations of biologically and immunologically active insulin. The nonpancreatic tumors had no significant concentration of insulin or insulinlike activity, but results were equivocal in one hepatoma. The mechanism by which such tumors induce hypoglycemia remains unknown. It does not appear to result from the elaboration of insulin or of products with insulinlike activity. DIABETES *22*:762-67, October, 1973.

Hypoglycemia associated with nonpancreatic tumors has been a recognized clinical entity for almost four decades. The mechanism by which the hypoglycemia is induced in this circumstance has not been determined, but increased amounts of circulating insulin or insulinlike activity and tumor concentrations of the hormone have been reported.[1-7] Additional postulations include excessive utilization of glucose by tumor tissue,[2,7-11] the elaboration of factors that block hepatic gluconeogenesis,[12-14] and adipose tissue lipolysis.[15]

We wish to report the results of assays for insulin performed with immunologic and biologic insulin assay methods on tissue extracts from ten nonpancreatic tumors associated with hypoglycemia and five pancreatic islet-cell adenomas.

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MATERIALS AND METHODS

Tumor tissue specimens were obtained from ten patients with nonpancreatic tumors associated with hypoglycemia and from five patients with pancreatic islet-cell adenomas. Control specimens were obtained during surgery in some patients from adjacent normal-appearing tissue or other tissue and during postmortem examination of seven presumably healthy young subjects who had died during automobile accidents. Control tissues included muscle, liver, kidney, adipose tissue, adrenal gland, hypophysis, thymus, and pancreas. Portions of tumors and adjacent tissue were collected immediately after surgical resection; the control tissue that derived from autopsies was obtained within six hours of death. All specimens, whether tumor or control, were frozen on dry ice within thirty minutes of excision.

Four tissue extraction solutions were utilized: acid alcohol,[16] normal saline, boracic-succinic acid buffer pH 5.3 and sodium phosphate buffer pH 7.0. Tissue was ground mechanically at 2° C. in each of the extraction solutions. All extraction yields were placed in washed Visking tubing and dialyzed against an infinite volume of distilled water at 2° C. The contents of the dialysis bags were then freeze-dried and stored on dry ice until insulin assays were performed.

On the day of assay for insulinlike activity (ILA), a 5 mg. per cent solution was made of each dried tissue extract in Krebs' bicarbonate buffer, and an aliquot of the solution was returned to frozen storage for later immunoassay (within four weeks). From the 5 mg. per cent solution, a 0.5 mg. per cent solution was made. ILA was determined on this dilution with a modification of the rat epididymal fat assay that utilizes the conversion of glucose 1-C-14 to C-14-labeled lipid as the index of insulin activity.[17] Immunoassays were performed by the double antibody technic of Morgan and Lazarow.[18] All resultant assay values have been expressed in milliunits per gram weight of original wet tissue. Because of inherent variability in the assay for

ILA by rat fat pad bioassay, multiple (6 to 43) determinations on the same tissue specimen were performed and single median values were used from each assay for final statistical analysis in order to reduce the risk of undue influence by extremes of bioassay value. Because variation in the immunoassay is relatively small, only three determinations on each specimen were performed with this method.

RESULTS

The results of the buffered extractions using pH 5.3 and pH 7.0 were identical to those obtained with normal saline, and for this reason, only the latter observations are recorded in the data.

Normal tissues. As shown in table 1, the median ILA concentration in seven nonpancreatic control tissues ranged from 8 to 396 milliunits per gram of wet tissue weight by acid alcohol extraction and from 32 to 616 milliunits per gram using normal saline extraction. Adipose tissue contained the lowest concentration of ILA, whereas the highest was associated with hypophysis. The mean of the median IRI concentration was 63 ± 38.7 and 16 ± 3.9 milliunits per gram wet tissue with the two extraction methods. Insulin assays were performed on multiple specimens from only two nondiabetic pancreases; their median IRI values were 358 and 169 with a mean of 264 milliunits per gram with acid alcohol extraction and 23 and 34 with mean of 29 milliunits per gram wet tissue with normal saline extraction (table 2). These two pancreases were obtained from two young patients who died in accidents; they allegedly had not had diabetes, but since glucose tolerance tests were not performed immediately before death, diabetes mellitus and chronic pancreatitis were not definitely excluded. This could account for the ILA and IRI tissue concentrations, which are lower than reported levels,[19-20] although within the range of values detected in individual patients.

Islet beta-cell adenomas. Insulin assays of five beta-cell adenomas revealed large quantities by both methods (table 2). Concentrations of IRI and ILA are expressed as units per gram wet tissue. In general, IRI concentrations were greater than ILA values in beta-cell adenomas, as contrasted with greater ILA than IRI levels in extracts of normal pancreatic tissue. In the latter,

TABLE 1

Insulin assays of nonpancreatic tissue extracts in seven normal adults

Tissue	Extraction solution	Biological insulin activity (ILA) (Milliunits per gram wet tissue)			Immunoreactive insulin (Milliunits per gram wet tissue)
		Number of determinations	Range of ILA values	Median	Median of three determinations
Muscle	Acidol	18	56-616	230	312
	Saline	24	92-966	267	7
Liver	Acidol	21	52-177	73	17
	Saline	21	252-958	554	25
Kidney	Acidol	18	64-602	96	54
	Saline	16	164-886	394	26
Adipose tissue	Acidol	18	4- 15	8	1
	Saline	18	20- 92	32	4
Adrenal	Acidol	18	87-418	162	26
	Saline	18	196-862	549	29
Hypophysis	Acidol	6	264-561	396	13
	Saline	6	431-1,568	616	19
Thymus	Acidol	18	70-385	196	17
	Saline	18	209-638	385	5
Mean ± S.E.M.	Acidol		85-396	166 ± 47.8*	63 ± 42†
	Saline		195-853	400 ± 76.4*	16 ± 4.1†

* Significantly different from each other when the two methods of extraction are compared ($p > .025$).
† NS $P = 0.30$

TABLE 2

Insulin assays of extracts of five islet-cell adenomas

Patient	Extraction solution	Biological insulin activity (ILA) (Units per gram wet tissue)			Immunoreactive insulin (Units per gram wet tissue)
		Number of determinations	Range of ILA values	Median	Median of three determinations
Po	Acidol Saline	18	13.7- 41.6	29.2	73.5
Sl	Acidol Saline	10 8	0.7- 6.5 2.6- 7.8	2.1 5.3	4.4 6.3
Li	Acidol Saline	10 8	1.6- 12.0 0.2- 1.8	3.6 0.8	2.6 1.4
Wh	Acidol Saline	8 10	12.0- 84.0 44.7-172.0	27.2 93.7	101.2 263.1
Du	Acidol Saline	43 24	1.9- 38.8 1.5- 9.5	8.6 3.7	9.0 0.2
Mean ± S.E.M.	Acidol Saline		6.0- 36.5 12.2- 47.7	14.1 ± 6.5 25.9 ± 11.3	38.1 ± 20.5 67.7 ± 65.1
Two pancreases presumed normal	Acidol Saline	40 40	0.7- 3.6 1.4- 9.1	1.6 4.4	0.26 0.03

ILA concentration in normal saline extraction were higher than those in acid alcohol extraction.

Nonpancreatic tumors. Insulin assays were performed on ten nonpancreatic tumors associated with hypoglycemia. Tissue samples were obtained from the primary tumor in all except one patient with Hodgkin's disease, in whom the tissue sample was obtained from a metastatic liver nodule. Tumors consisted of two adrenal carcinomas, three hepatomas, one melanoma, one bronchogenic carcinoma, one breast carcinoma, one liver nodule of Hodgkin's disease, and one sarcoma. The results are shown in table 3.

The assay content of the two adrenal carcinomas was indistinguishable from that of normal adrenal gland and normal nonpancreatic tissues. Insulin assay results of the three hepatomas were variable. ILA levels were in the same range as those of normal liver and the nonneoplastic hepatic tissue of the same patient. In one patient (U.N.), the IRI concentration was greater in the hepatoma than in control tissue, but it was within the range detected in presumably normal pancreatic tissue. The assay values of other tumors in the table were not greater than those of normal tissues.

In general, the ILA yield of nonpancreatic tumors and control tissues was greater than the IRI yield with both acidol and saline extraction except in muscle (tables 1 and 3). ILA and IRI concentrations of nonpancreatic tumors associated with hypoglycemia, when compared to those of islet-cell adenomas, were much reduced and when compared to normal nonpancreatic tissues, were not significantly higher by either biologic or immunologic assay, except in one case of hepatoma (U.N.), in which the results were equivocal (table 4).

DISCUSSION

The mechanism by which nonpancreatic tumors are associated with hypoglycemia remains to be determined. Earlier reports suggested that secretion of an insulin or insulinlike substance by some of these tumors[1-7] might be responsible, but others have challenged this explanation. Alternative explanations include release of a substance which stimulates insulin production by pancreatic islets,[21] impairment of hepatic glucose output by extensive tumor metastasis,[22] or excessive utilization of glucose by the tumor.[2,7-11] Recently other workers have postulated the elaboration of a substance by the tumor capable of blocking gluconeogenesis,[12-14] hepatic glycogenolysis,[12,15,23] and adipose tissue lipolysis.[15] Also, the suggestion has been made that tumor production of tryptophan or its metabolites could inhibit gluconeogenesis and thereby lead to hypoglycemia.[24-26]

TABLE 3

Insulin assays of extracts of ten nonpancreatic tumors and nonneoplastic tissues of same patients

Tumor or tissue	Extraction solution	Biological insulin activity (ILA) (Milliunits per gram wet tissue)			Immunoreactive insulin (Milliunits per gram wet tissue)
		Number of assay determinations	Range of ILA values	Median	Median of three determinations
Adrenal carcinoma #1	Acidol	18	104- 312	187	80
	Saline	18	104- 364	187	4
Adrenal carcinoma #2	Acidol	19	106- 678	138	0
	Saline	16	170- 748	204	0
Hepatoma (G.H.)	Acidol	20	50- 310	110	52
	Saline	16	131- 566	246	66
Hepatoma (J.B.)	Acidol	16	122- 374	216	5
	Saline	18	225- 960	525	11
Hepatoma (U.N.)	Acidol	15	207- 830	451	392
	Saline	18	237-1,226	423	51
Sarcoma (D.H.)	Acidol	18	42- 193	92	1
	Saline	18	134- 683	220	2
Melanoma	Acidol	18	80- 208	120	27
	Saline	18	132- 352	282	41
Bronchial tumor	Acidol	18	30- 162	54	3
	Saline	18	110- 550	231	1
Breast carcinoma	Acidol	10	58- 174	128	1
	Saline	6	86- 473	86	1
Liver nodule Hodgkin's disease	Acidol	10	90- 306	216	2
	Saline	8	90-1,530	135	1
Mean ± S.E.M.	Acidol		89- 355	171 ± 35.2	56 ± 38
	Saline		142- 745	254 ± 39.4	18 ± 8
Liver (D.H.)	Acidol	18	95- 435	163	0
	Saline	18	286-1,716	658	2
Spleen (D.H.)	Acidol	18	116- 650	209	2
	Saline	18	234- 655	304	1
Heart (D.H.)	Acidol	18	51- 212	115	5
	Saline	18	437-1,081	667	3
Liver (J.B.)	Acidol	18	76- 342	137	1
	Saline	18	174- 464	290	1
Liver (U.N.)	Acidol	10	100- 480	150	35
	Saline	18	14- 350	182	3
Mean ± S.E.M.	Acidol		88- 424	155 ± 15.5	9 ± 7.1
	Saline		229- 853	420 ± 101	2 ± 0.4

Our observations are concerned with one aspect of the problem: the possibility that nonpancreatic tumors elaborate a noninsulin product with insulinlike activity. We employed several extraction methods to avoid limitations that might attend a single procedure. We also used two types of control tissue by comparing tumor insulin concentration with adjacent noninvolved tissue (tissue to which the tumor belonged embryologically) in some patients and with tissues from presumably healthy young subjects who died in accidents.

The results do not support the hypothesis that the tumors elaborated a noninsulin polypeptide or other nondialyzable product with insulinlike activity. The nonpancreatic tumors evaluated contained negligible levels of ILA and IRI, except that IRI values were high in one hepatoma (U.N.), as compared with values in

TABLE 4

Comparison of insulin concentrations in normal tissues, nonpancreatic tumors; and islet-cell adenomas (Summary of tables 1, 2, and 3)

Tissue	Extraction solution	Biological insulin activity (ILA) (Mean ± S.E.M. of median values) Milliunits/gm. wet tissue	Immunoreactive insulin (Mean ± S.E.M. of median values) Milliunits/gm. wet tissue
Normal tissues	Acidol	166 ± 47.8	63 ± 38.7
	Saline	400 ± 76.4	16 ± 3.9
Normal tissue of patients with nonpancreatic tumors	Acidol	155 ± 15.5	9 ± 7.1
	Saline	420 ± 101	2 ± 0.4
Nonpancreatic tumors	Acidol	171 ± 35.2	56 ± 38
	Saline	254 ± 39.4	18 ± 8
Islet-cell adenomas	Acidol	14,165 ± 6,539	38,177 ± 20,550
	Saline	25,908 ± 11,314	67,794 ± 65,136

control and pancreatic tissue. The mechanism by which most of extrapancreatic tumors induced hypoglycemia does not appear to result from the elaboration of insulin or a similar peptide by the tumor. However, tissue concentrations do not necessarily reflect secretion rates, which have not been evaluated in this study. They could have been high and caused hypoglycemia in spite of normal residual tissue concentrations.

It is not clear from available observations why some islet-cell adenomas contain more assayable IRI than ILA (table 2). Perhaps high concentrations of proinsulin in some insulinomas[27,28] or in sera of patients with insulinoma[29-31] account for the discrepancy, since proinsulin has a weak biologic effect but is partially detectable by immunoassay. Alternatively, nonspecific proteolysis may have taken place in the handling and extraction process, producing small, broken pieces of insulin, biologically inactive, but immunoreactive. Finally, nondialyzable tissue factors with an insulin antagonistic effect could have come through the extraction procedure to reduce the net insulin effect in the epididymal fat pad assay.

The lack of assayable insulin in the tumor extracts of the present study does not completely exclude the possibility that an insulinlike factor is elaborated by nonpancreatic tumors. It might have been denatured by the extraction process or lost during dialysis. The greater yield of insulin activity in tumor extracts by normal saline, for example, may indicate that acid alcohol has a deleterious effect on a molecule with insulinlike activity. Possibly also, some serum cofactor is necessary for the postulated substance to exert its insulin activity in vivo; however, an assay of extract from sarcoma (D.H.) added to normal serum and incubated for two hours at 37° C. yielded no more insulin activity than the extract itself. Other experimental avenues will have to be developed if the pathogenesis of nonpancreatic tumor hypoglycemia is to be explained.

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