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Research Article

THE INTERACTION OF GLUCAGON AND INSULIN ON BLOOD GLUCOSE

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In previous publications from this laboratory (1, 2) evidence was presented which indicates that glucagon enhances the peripheral utilization of glucose [1] in normal men and dogs as well as in the depancreatized dog. The purpose of this paper is to report experiments on the combined effect of glucagon and insulin on arterial and venous glucose levels in normal men, and to compare the magnitude of this effect with that observed in subjects given similar amounts of each hormone separately.

METHODS

Fifty-six men (ages 21 to 47) considered normal with respect to carbohydrate metabolism and in good general health and nutritional state were studied. Controlled basal conditions were obtained in the following manner: (a) Subjects were hospitalized and placed on a high carbohydrate (360 gm.), high caloric diet (3500 calories) for at least three days before being studied; (b) a 14-hour fast preceded each study; (c) experiments were begun at 8 a.m. following a 30 to 60-minute period of bed rest in the test bed in a special room; (d) venous blood samples were obtained from an indwelling needle inserted (using local Procaine anesthesia) five minutes before the experiment was begun; (e) every effort was made to keep the subjects motionless, relaxed and comfortable during the experiments.

Ten per cent glucose was infused intravenously at a constant rate (250 to 370 mg. per min. in different subjects) throughout the test period by means of a Bowman constant infusion pump. The initial 30 to 60 minutes of the infusion period served as a control period, following which glucagon [2] and/or insulin [2] was added to the infusion bottle. The glucagon dosage was 0.7 to 1.0 mg. administered by constant intravenous infusion over periods ranging from 60 to 100 minutes. Insulin dosage ranged from 0.054 to 0.128 units per min. by constant intravenous infusion for periods ranging from 65 to 110 minutes. Mean insulin dosage for the subjects given insulin alone was .076 units per minute, for the subjects given insulin plus glucagon it was .071 units per minute. The dosage of insulin was arrived at by trial and error; the criteria used were that the dose be sufficient to cause a definite drop in blood sugar, but not so large as to result in hypoglycemic values. Data in which arterial blood sugar fell below 65 mg. per cent were discarded. In the subjects who received both insulin and glucagon, the insulin infusion was begun 15 to 25 minutes before the glucagon in order to obtain the maximum effect of each hormone (on blood sugar level) at approximately the same time.

Venous and capillary (finger) blood specimens were drawn at five or ten-minute intervals, a total of 30 to 40 samples during each experiment. Venous samples were obtained from the antecubital vein which carries mixed blood from the deep and superficial tissue of the forearm and hand (3, 4). Capillary blood contains the same glucose concentration as arterial blood (5). Samples were analyzed for glucose content by the Nelson-Somogyi method (6). Sodium fluoride and immediate refrigeration were used to prevent glycolysis in the samples. Capillary samples were analyzed singly, venous samples in duplicate. In approximately 900 duplicate venous samples the maximum difference between any pair was 10 per cent, and the average was 2 per cent.

The A-V/A data were subjected to a statistical analysis by means of a non-parametric median test (7); *i.e.*, using the medians as a cutting point. The data were placed in a 2×2 contingency table, and then subjected to a chi-square analysis.

RESULTS

The data on the 12 subjects who received insulin following an initial control period are given in Table I. The data from one of these experiments are shown in Figure 1. In 11 of the 12 subjects insulin resulted in a fall in arterial and venous blood glucose levels to or below fasting levels in spite of the constant glucose infusion.

[1] Glucose utilization is used to mean the disappearance of blood glucose.

[2] Generously supplied by Drs. O. K. Behrens and W. R. Kirtley of Eli Lilly Company. The glucagon (Lot 208-158B-214A) contains 50 per cent of the potency of the crystalline standard and an insulin content of 0.05 to 0.005 units per milligram. The insulin (Lot No. T-3206) was a preparation free of glucagon.

TABLE I

Insulin cases *

Patient Wt. (Kg.)	Age	Glucose mg./min.	Insulin units/min.		Time in minutes 0	10	20	30	40	50	60	70	80	90	100	110	120	130
W. Y. 77.8	22	344	.128	A†	89	108	108	125	130	134 ↑	136	136	127	118	106	92		
				V†	87	105	105	123	128	132 |	135	130	121	111	99	85		
E. J. 79	25	360	.088	A	86	105	114	120	124	127 ↑	129	130	128	117	99	85	77	
				V	78	100	108	112	115	116 |	117	117	114	105	93	82	74	
R. B. 81.6	29	360	.113	A	97	109	120	129	134	138 ↑	140	135	122	106	90	76		
				V	92	104	115	122	127	131 |	133	127	116	104	91	78		
C. J. 70.7	26	370	.061	A	87	102	116	126	134	140 ↑	144	146	146	141	135	127	122	
				V	82	98	109	117	123	127 |	130	132	133	133	132	125	117	
R. G. 69.7	36	360	.082	A	89	109	120	127	131	133 ↑	134	135	135	122	109	97	87	
				V	88	105	114	121	125	128 |	130	131	127	113	101	90	81	
J. C. 70.5	32	295	.069	A	93	123	138	149 ↑	150	139	118	98	82					
				V	91	113	126	136 |	138	113	92	80	71					
P. W. 67	21	270	.073	A	100	117	128	134 ↑	134	129	120	108	97	88	79	72	70	
				V	95	109	118	123 |	122	111	94	85	80	75	71	68	66	
J. H. 61.3	21	270	.061	A	96	111	122	130 ↑	134	134	132	126	118	108	100	94		
				V	92	105	115	122 |	125	122	110	101	92	84	76	69		
A. M. 88	27	250	.054	A	92	100	106	110 ↑	110	109	106	101	97	93	88	83	77	
				V	83	92	98	102 |	103	99	94	88	83	78	74	69	65	
R. S. 71	32	300	.059	A	94	117	133	142 ↑	146	146	146	146	142	136	128	118	108	97
				V	93	110	124	134 |	139	139	139	139	136	130	123	112	98	82
W. H. 58.6	24	300	.061	A	85	112	128	137 ↑	142	141	137	129	119	108	95	84	76	
				V	82	101	116	126 |	132	134	126	113	104	88	78	71	63	
K. W. 74	38	260	.069	A	84	105	113	118 ↑	122	125	124	121	115	108	102	97	92	88
				V	85	101	108	108 |	105	98	90	83	77	71	66	61	58	55

* Blood sugar values at 10-minute intervals taken from curves constructed from determinations on samples (30 to 40 per subject) drawn at staggered time intervals which varied in the different subjects.
† A—Capillary blood sugar (mg. per cent). V—Antecubital vein sugar (mg. per cent).
↑ Beginning of insulin infusion.

A-V/A values [3] with insulin were significantly increased over control values ($P = .01$).

The data on the 12 subjects who received a combined infusion of insulin and glucagon after an initial control period are shown in Table II and one of the experiments is plotted in Figure 2. In eight subjects of this group there was a marked arterial hyperglycemia (over 200 mg. per cent) when glucagon was added to the glucose-insulin infusion mixture; in three there was little or no increase in arterial blood sugar over control values. In 11 of the 12 subjects a marked increase in A-V glucose differences occurred (see Table II). The three subjects who showed little or no arterial hyperglycemia after the addition of glucagon were, nevertheless, among those who exhibited marked increases in A-V glucose differences.

A comparison of the data of the insulin plus glucagon group with the groups who received glucagon (previously reported [2]) or insulin alone revealed the following noteworthy differences: (a) the A-V/A values for the insulin plus glucagon group were significantly greater ($P = .001$) than those for either of the other two groups (Figure 3); (b) the arterial hyperglycemia of the insulin plus glucagon group was often greater than that of the glucagon group despite the fact that the mean glucose infusion rates [4] of the former

[3] A-V/A = arterio-venous glucose difference divided by arterial glucose concentration. This value has been shown to be proportional to the specific rate constant of peripheral glucose utilization under the special conditions of the experiments (2).

[4] The mean glucose infusion rates (mg. per minute) for the three groups were: glucagon 321; insulin 312; and insulin plus glucagon 296.

were slightly lower than those of the latter, and (c) the fall in blood sugar below initial fasting levels so commonly observed in the insulin group did not occur [5] in the insulin plus glucagon group.

DISCUSSION

That insulin enhances the peripheral utilization of glucose is now widely accepted. The recent demonstration (2) that glucagon has a similar action which is independent of a secondary release of insulin has already been cited. The experiments reported here indicate that when glucagon and insulin are administered together peripheral glucose utilization is increased beyond that with either hormone alone. In addition, the opposing effect of the two on arterial blood sugar may result, at times, in a virtually unchanged level. The greater arterial hyperglycemia often observed with glucagon plus insulin as compared with glucagon alone may have resulted from higher liver glycogen levels induced by the insulin which was administered 15 to 25 minutes before the glucagon in the subjects who received both hormones.

These findings suggest that insulin and glucagon are capable of functioning jointly to enhance the peripheral utilization of glucose and at the same time, of counteracting the opposing effect of each other on the blood sugar. Judging from the variation in responses observed in different subjects

[5] The only exceptions are the last two venous values on subject T. H.

TABLE II

Insulin + glucagon cases *

Patient Wt. (Kg.)	Age	Glucose mg./min.	Insulin units/min.		Time in minutes 0	10	20	30	40	50	60	70	80	90	100	110	120	130	140
H. C.	33	370	.086	A‡	86	103	114	121	126	130	133 ↑	134	136 ↑↑	158	164	163	151		
71.8				V‡	83	98	109	116	121	124	127 |	127	128 ||	141	137	133	129		
D. D.†	39	316	.111	A		130	136	141 ↑	145	138	127 ↑↑	128	140	133	126	119	114		
68.1				V		124	129	133 |	136	126	106 ||	105	110	110	105	99	96		
M. R.†	27	333	.065	A		149	150	153 ↑	161	162 ↑↑	175	200	212	209	189	174	165		
66.4				V		132	134	136 |	141	145 ||	138	153	159	155	147	137	126		
T. D.	37	265	.062	A	106	128	143	150 ↑	155	157 ↑↑	184	208	220	222	217	208	197	185	172
68.4				V	100	118	132	140 |	145	140 ||	142	145	142	138	136	138	145	138	110
J. G.	26	275	.084	A	100	108	121	130 ↑	135	140 ↑↑	163	189	201	197	183	167	157	149	134
53.8				V	95	100	112	121 |	127	132 ||	145	171	186	184	171	160	152	144	128
E. H.	37	270	.058	A	93	110	121	126 ↑	129	129 ↑↑	136	163	172	172	159	145	132	122	113
73.4				V	91	104	113	118 |	120	120 ||	123	141	143	137	128	119	110	103	100
A. O.	43	320	.064	A	88	100	110	119 ↑	125	135 ↑↑	166	193	213	219	212	205	202	197	176
73.6				V	85	96	105	114 |	119	124 ||	136	155	167	174	171	154	148	153	147
C. R.	23	310	.054	A	82	101	115	124 ↑	129	130 ↑↑	161	198	213	201	192	184	177	170	163
71				V	82	101	109	113 |	114	115 ||	110	136	140	137	122	116	121	120	116
R. R.	29	290	.062	A	95	113	121	127 ↑	131	135 ↑↑	146	185	199	203	185	170	160	151	137
75				V	92	106	114	120 |	124	128 ||	116	163	187	197	181	167	156	147	134
J. C.	34	290	.061	A	90	108	121	129 ↑	133	135 ↑↑	163	189	202	197	190	184	180	177	176
80.5				V	90	101	112	121 |	127	121 ||	133	145	150	151	150	149	147	147	151
T. H.	38	250	.065	A	92	111	119	123 ↑	126	128 ↑↑	149	167	166	151	138	126	118	107	91
75.5				V	92	104	112	117 |	119	121 ||	122	136	140	123	113	105	97	85	71
J. S.	38	300	.077	A	98	122	141	154 ↑	161	158 ↑↑	179	213	227	230	229	224	212	199	198
79				V	92	113	128	137 |	141	141 ||	154	170	174	173	173	180	184	185	183

* Blood sugar values at 10-minute intervals taken from curves constructed from determinations on samples (30 to 40 per subject) drawn at staggered time intervals which varied in the different subjects.

† Control period data not used for statistical analysis because a loading dose of glucose was used.

‡ A—Capillary blood sugar (mg. per cent). V—Antecubital vein sugar (mg. per cent).

↑ Beginning of insulin infusion. ↑↑ Beginning of glucagon infusion.

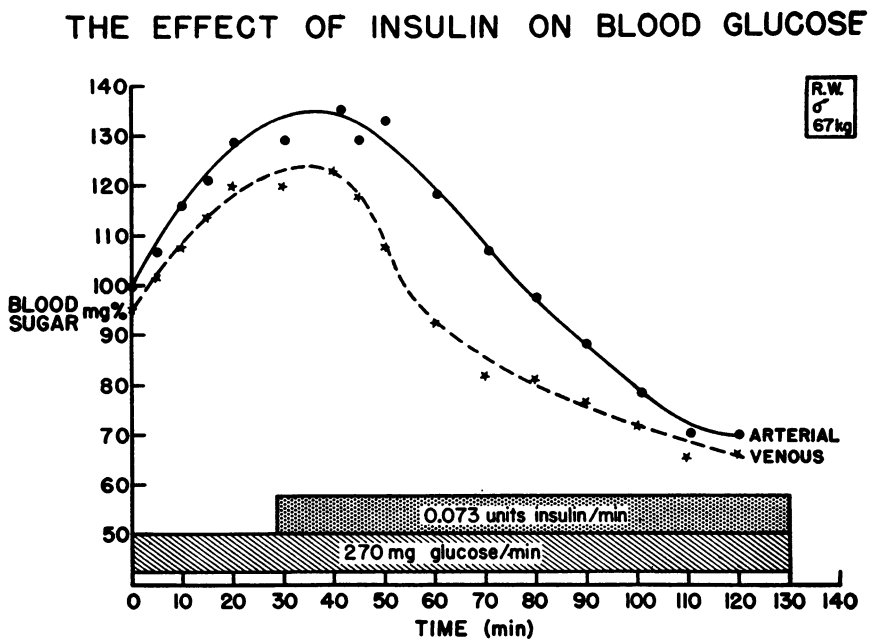

FIG. 1. THE EFFECT OF A CONTINUOUS INTRAVENOUS INFUSION OF INSULIN ON ARTERIAL AND VENOUS BLOOD SUGAR LEVELS IN A NORMAL MAN

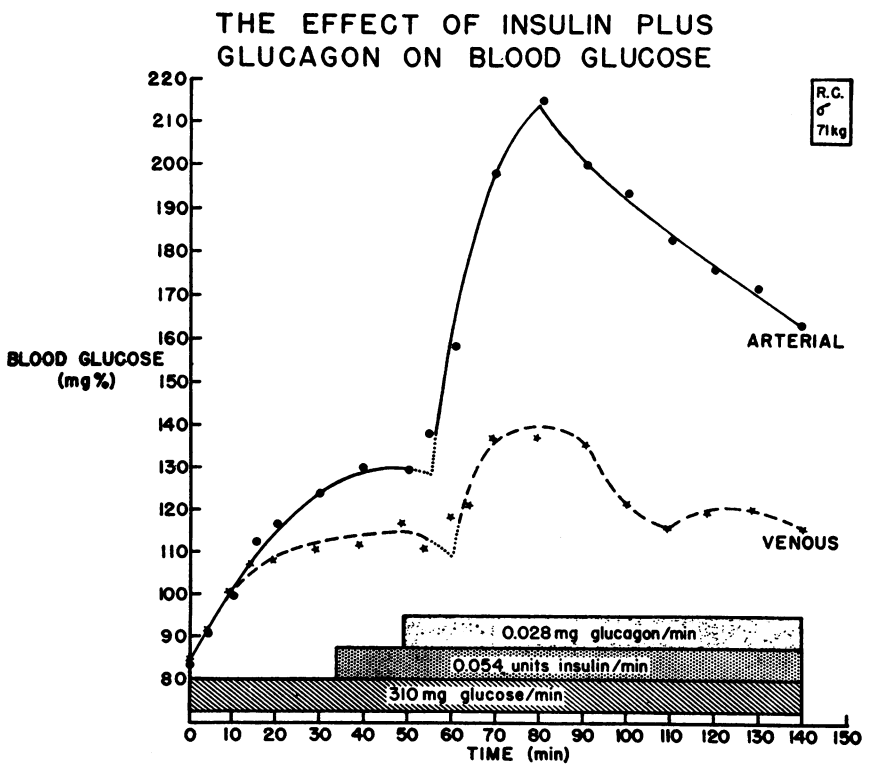

FIG. 2. THE EFFECT OF A CONTINUOUS INTRAVENOUS INFUSION OF GLUCAGON PLUS INSULIN ON ARTERIAL AND VENOUS BLOOD SUGAR LEVELS IN A NORMAL MAN

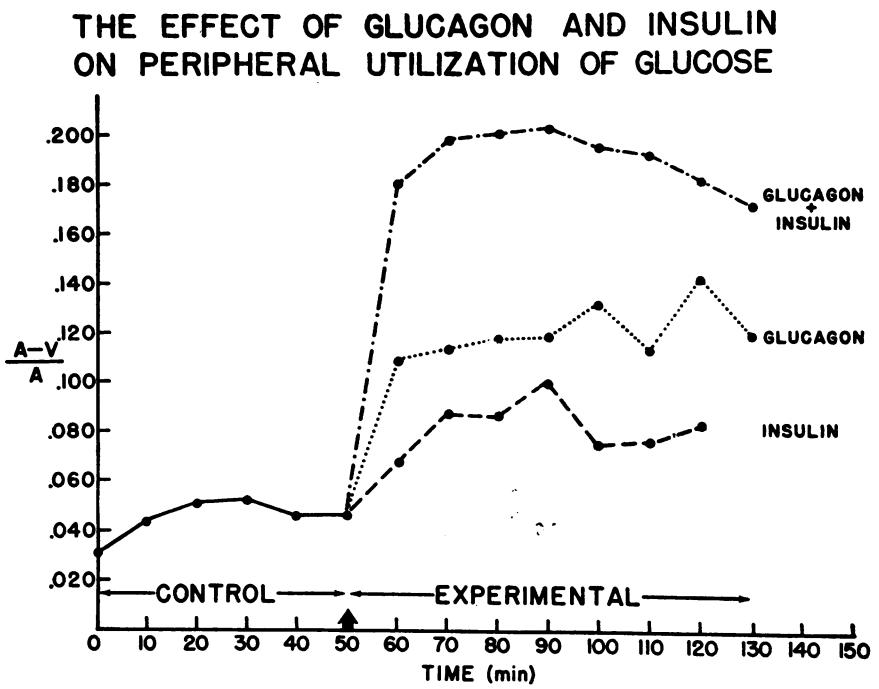

FIG. 3. A SUMMARY OF CONTINUOUS INTRAVENOUS INFUSION EXPERIMENTS IN NORMAL MAN SHOWING THE DIFFERENCE BETWEEN THE EFFECT OF INSULIN, GLUCAGON, AND GLUCAGON PLUS INSULIN ON AN INDEX OF THE PERIPHERAL UTILIZATION OF GLUCOSE (A-V/A)

Each point of the control period represents the median value of 54 subjects, whereas each point of the three experimental groups represents the median value of 12 subjects. The data on the glucagon subjects were taken from a previous publication (2).

receiving similar doses, there is considerable individual difference in responsiveness to the two hormones. No correlation of response with body weight was observed.

The fact that glucagon and insulin have opposing actions on blood glucose and liver glycogen levels has led many to postulate that they are antagonists (8, 9). Another concept of glucagon's role has been advanced by Bürger (10) and by Pincus and Rutman (11). These workers suggest that glucagon acts as an adjunct to insulin because it mobilizes liver glycogen for the enhancing action of insulin on glucose utilization in the peripheral tissues.

It has been reported that glucagon inhibits the action of insulin on glucose uptake by the isolated rat diaphragm (12). This was a preliminary report on work done before purified preparations of glucagon were available. Smith in F. G. Young's laboratory was unable to confirm these results (13). Ingle, Nezamis, and Humphrey observed no effect of glucagon on the disposal of administered glucose in the eviscerated rat (14). Drury, Wick, and Sherill reported that in the eviscerated rabbit glucagon had no effect on glucose oxidation but had a slight inhibiting effect on the disappearance of blood glucose (15). The apparent discrepancy between some of these findings and those reported here might be explained by the studies of Lang, Goldstein, and Levine (16). These workers have presented evidence suggesting that the liver contains a "humoral agent" which enhances peripheral glucose utilization even in the presence of maximal insulin dosage. It is conceivable that glucagon causes the release of this liver factor and that this accounts for the difference between the results in the normal human as compared with the eviscerated animal and the isolated rat diaphragm. In keeping with this hypothesis is the observation that glucagon has no effect on blood sugar levels or arterio-venous glucose differences in the hepatectomized dog (17).

SUMMARY

Studies on the effect of glucagon and insulin on the disappearance of blood glucose in 56 normal men using a constant glucose infusion technique are reported. The results indicate that the two hormones administered together (a) cause a significantly greater increase in peripheral glucose utilization than either one alone, and (b) may result in a neutralization of the opposing action of each other on arterial blood sugar levels. These findings suggest that glucagon and insulin may function jointly to regulate the level of blood glucose and the utilization of carbohydrate by peripheral tissues.

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