BBA 3934

THE INFLUENCE OF INTRAVENOUS GLUCOSE ON BLOOD-INSULIN ACTIVITY, AND ALSO ON THE GLUCOSE UPTAKE OF THE DIAPHRAGM AND OF THE ADIPOSE TISSUE OF RATS

M. C. GARCIA-FERNANDEZ* AND J. L. R-CANDELA
Instituto "G. Marañón", Madrid (Spain)
(Received June 6th, 1962)

SUMMARY

1. After the intravenous injection of glucose into normal male rats, we have measured the plasma-insulin activity, the glucose uptake of the diaphragm and of epididymal adipose tissue from 5–90 min after the injection. We have observed an increased uptake of glucose by the diaphragm and a rise of insulin in the blood 10 min after the injection. In the adipose tissue we have observed an inhibition of glucose uptake at all times up to 60 min.

2. 10 min after the injection of L-leucine the blood insulin rises and there is an increase in the uptake of glucose by the diaphragm and by the epididymal adipose tissue.

3. The inhibition of uptake of glucose by adipose tissue, observed after intravenous injection of glucose, is not produced in adrenalectomized rats. It is suggested that hyperglycemia produces secretion of glucocorticoids which are responsible for the inhibiton of the uptake of glucose in adipose tissue.

INTRODUCTION

In 1952 PERLMUTTER, WEISENFELDT AND MUFSON[1] found no evidence of increased blood-insulin activity after the intravenous injection of glucose, CANDELA, ROVIRA AND CANDELA[2,3], however, were able to demonstrate, in dogs, that the hyperglycemia produced by this means gives rise to a rapid increase of insulin in the blood, which recedes shortly afterwards, to reappear some time later. WHITNEY AND YOUNG[4] studied the plasma-insulin activity and the glucose uptake of rat diaphragm after the intravenous injection of glucose. They found an activity less than normal 30 min after the injection, the glucose uptake first increasing and later falling below the normal.

Bearing in mind the foregoing results, the question arises whether hyperglycemia results in the release of insulin only, with muscle as one of its destinations, or whether it also produces secretion of other hormones whose target may be different tissues.

* Part of Doctoral Thesis.

MATERIAL AND METHODS

Male Wistar rats, from our colony, of 120–160 g weight, were kept 16–20 h in a dark room, without noise, and at 20°, either with free access to food or fasting and with free access to water.

In the case of adrenalectomized rats the adrenalectomy and post-operative care was according to the usual practice. When the wound was cicatrized and the animal completely recovered, the effect of an injection of glucose was investigated in the same manner as with normal rats.

At the times after injection of either glucose or L-leucine indicated in the section RESULTS the rats were killed by decapitation. The blood was heparinized for the determination of plasma insulin as described in another paper[5]. Immediately after collecting the blood, the diaphragm and the adipose tissue of the epididymis of each animal were removed. The hemidiaphragms (200 ± 5 mg) and the adipose tissue (200 ± 5 mg) were incubated in Warburg vessels, charged with Krebs–Henseleit buffer and containing 3 mg of glucose/ml at 37.5°. The time for the determination of the uptake of glucose by the diaphragm was 1.5 h, for that of the epididymal adipose tissue, 5 h. Glucose was estimated by the Somogyi–Nelson method.

RESULTS

The experiments were carried out in three groups. In the first group, the effect of intravenous injection of glucose on the glucose uptake of diaphragm and of epididymal adipose tissue, and also on the level of insulin in the plasma was assessed over a time course of 90 min. In the second group, the effects were studied 10 min after the intravenous injection of L-leucine. In the third, the effect of adrenalectomy was studied.

The results of the first group of experiments are shown in Table I and represented graphically in Fig. 1. It can be seen that injection of glucose produces a significant increase in the uptake of glucose by the diaphragm, compared with non-injected controls, 10 and 15 min after the injection. In the adipose tissue, there is a significant decrease in the glucose uptake at all times studied, except at 60 min. The level of insulin in the plasma is raised significantly only at 10 min, after which the values remain normal up to 90 min. From this we may infer that the injection of glucose produces an increased secretion of insulin, as observed previously[2,3,4].

In Table II are shown the results obtained 10 min after the injection of L-leucine from which it is clear that this amino acid produces a significant increase both of plasma insulin and of glucose uptake in diaphragm and adipose tissue. Comparison of the effects of glucose and of L-leucine administration suggests that when the secretion of insulin is not produced by hyperglycemia, this hormone is bound both by muscle and by adipose tissue, thus facilitating the passage of glucose into the interior of the cell in both tissues.

Table III shows the results obtained 10 min after injection of glucose into adrenalectomized male rats. The glucose uptake of adipose tissue, and of diaphragm, is greater in the adrenalectomized rats than in the normals and the plasma insulin is higher. Injection of glucose does not result in a lowering of the uptake of glucose, as in the adipose tissue of normal animals, but in a significant increase in all three parameters.

TABLE I

THE EFFECT OF INTRAVENOUS INJECTION OF 200 mg OF GLUCOSE INTO NORMAL RAT

Insulin activity is expressed in terms of the increase in glucose uptake by diaphragm incubated in diluted plasma, as described previously[5]. N.S., difference between control and experimental values not significant.

Time after intravenous injection of glucose (min)	*Glucose uptake by diaphragm in vitro (mg/g/90 min)**	*Change in glucose uptake by diaphragm in vitro (mg/g/90 min)*		*Glucose uptake by epididymal fat in vitro (mg/g/5 h)**	*Change in glucose uptake by epididymal fat in vitro (mg/g/5 h)*		*Plasma insulin (diaphragm method) (mg/g/90 min)*		
5	7.78 ± 0.33 (20) 8.05 ± 0.32 (20)	−0.27	N.S.	5.23 ± 0.35 (39) 6.86 ± 0.36 (39)	−1.63	$P < 0.01$	2.07 ± 0.15 (21) 2.22 ± 0.20 (20)	−0.15	N.S.
10	3.88 ± 0.10 (48) 3.50 ± 0.16 (32)	+0.38	$P < 0.05$	3.00 ± 0.08 (59) 3.54 ± 0.18 (44)	−0.54	$P < 0.01$	2.20 ± 0.17 (22) 1.58 ± 0.13 (21)	+0.62	$P < 0.01$
15	10.18 ± 0.52 (67) 7.84 ± 0.33 (67)	+2.34	$P < 0.01$	5.78 ± 0.31 (46) 5.44 ± 0.23 (55)	+0.34	N.S.	1.82 ± 0.19 (20) 1.42 ± 0.18 (19)	+0.40	N.S.
30	3.52 ± 0.21 (22) 3.90 ± 0.29 (21)	−0.38	N.S.	1.80 ± 0.13 (18) 2.57 ± 0.11 (21)	−0.77	$P < 0.01$	2.19 ± 0.38 (12) 1.76 ± 0.35 (10)	+0.43	N.S.
60	3.24 ± 0.14 (24) 3.36 ± 0.15 (24)	−0.12	N.S.	2.89 ± 0.23 (23) 1.99 ± 0.16 (24)	+0.90	$P < 0.01$	1.68 ± 0.34 (9) 1.39 ± 0.23 (11)	+0.29	N.S.
90	[illegible] ± 0.31 (30) 8.10 [illegible] 2 (28)	−0.27	N.S.	5.25 ± 0.29 (59) 6.38 ± 0.29 (56)	−1.13	$P < 0.01$	2.10 ± 0.25 (24) 1.59 ± 0.20 (25)	+0.51	N.S.

* All values are means ± standard errors. The values in parentheses represent the number of experiments.

TABLE II

COMPARISON OF THE EFFECTS OF THE INTRAVENOUS INJECTION OF GLUCOSE (200 mg) AND OF L-LEUCINE (11 mg/100 g BODY WEIGHT) INTO THE NORMAL RAT

The assays were carried out 10 min after the injections.

Substance injected	*Glucose uptake by diaphragm in vitro (mg/g/90 min)**	*Change in glucose uptake by diaphragm in vitro (mg/g/90 min)*		*Glucose uptake by epididymal fat in vitro (mg/g/5 h)**	*Change in glucose uptake by epididymal fat in vitro (mg/g/5 h)*		*Plasma insulin (diaphragm method) (mg/g/90 min)**		
Glucose	3.88 ± 0.10 (48) 3.50 ± 0.16 (32)	+0.38	$P < 0.05$	3.00 ± 0.08 (59) 3.54 ± 0.18 (44)	−0.54	$P < 0.01$	2.20 ± 0.17 (22) 1.58 ± 0.13 (21)	+0.62	$P < 0.01$
L-Leucine	5.81 ± 0.30 (20) 4.75 ± 0.30 (20)	+1.06	$P < 0.02$	3.22 ± 0.12 (38) 2.78 ± 0.17 (38)	+0.44	$P < 0.02$	2.83 ± 0.33 (10) 1.46 ± 0.44 (12)	+1.37	$P < 0.02$

* All values are means ± standard errors. The values in parentheses represent the number of experiments.

TABLE III

THE EFFECT OF THE INTRAVENOUS INJECTION OF 200 mg GLUCOSE INTO NORMAL AND ADRENALECTOMIZED RATS

The assays were carried out 10 min after the injection.

	*Glucose uptake by diaphragm in vitro (mg/g/90/min)**	*Change in glucose uptake by diaphragm in vitro (mg/g/90 min)*		*Glucose uptake by epididymal fat in vitro (mg/g/5 h)**	*Change in glucose uptake by epididymal fat in vitro (mg/g/5 h)*		*Plasma insulin (diaphragm method) (mg/g/90 min)*		
None	3.88 ± 0.10 (48) 3.50 ± 0.16 (32)	+0.38	$P < 0.05$	3.00 ± 0.08 (59) 3.54 ± 0.18 (44)	−0.54	$P < 0.01$	2.20 ± 0.17 (22) 1.58 ± 0.13 (21)	+0.62	$P < 0.01$
Adrenalectomy	9.56 ± 0.30 (21) 8.21 ± 0.29 (13)	+1.35	$P < 0.01$	6.83 ± 0.40 (24) 5.43 ± 0.42 (21)	+1.40	$P < 0.02$	4.14 ± 0.57 (13) 2.37 ± 0.34 (10)	+1.77	$P < 0.02$

* All values are means ± standard errors. The values in parentheses represent the number of experiments.

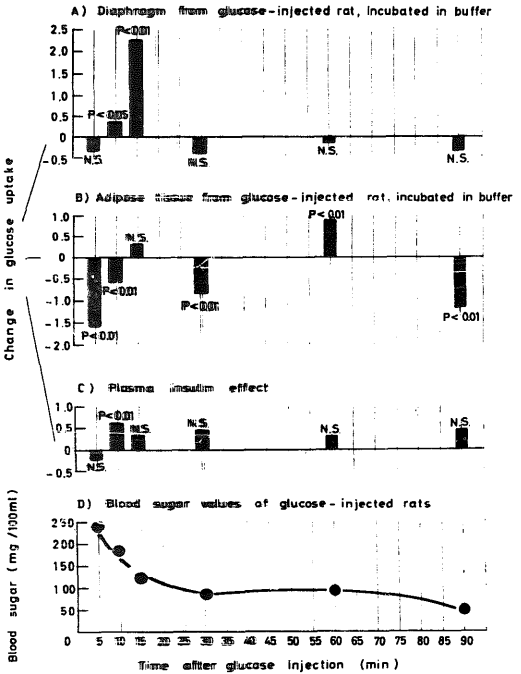

Fig. 1. Effect of intravenous injection of glucose on the glucose uptake of isolated diaphragm (A), and of adipose tissue (B) and also on the plasma-insulin effect (C). Blood-sugar levels at removal of tissue, shown in D

DISCUSSION

These results confirm that hyperglycemia produces an increase in the secretion of insulin. The greater uptake of glucose by the isolated diaphragm of the rat, removed after injection, first demonstrated by WHITNEY AND YOUNG, may be due to the binding of insulin, released from the pancreas, by muscle; the insulin could then exercise a stimulating effect on the uptake of glucose *in vitro*. This effect is, however, not observed in the adipose tissue, despite the greater insulin sensitivity of this tissue compared with that of striated muscle. As can be seen in Table I, except at 60 min after injection, the effect observed is an inhibition of the uptake of glucose. This inhibition is not produced if the stimulus for insulin secretion is L-leucine, (assuming that this amino acid elicits an increased secretion of this hormone) (Table II), or if hyperglycemia is produced in adrenalectomized animals (Table III). These facts lead us to conclude that the injection of glucose not only produces secretion of insulin, which

increases the uptake of glucose by the diaphragm and raises the level of insulin in the plasma, but also stimulates the adrenal gland. The latter effect gives rise to a secretion of glucocorticoids which produce the inhibitory effect on the glucose uptake observed in the adipose tissue.

The adipose tissue is considered to be the most probable site of action of the glucocorticoids. RIET-CORREA, MAGALHAES AND KRAHL[6] have observed that the stimulation by insulin, *in vitro*, of glucose uptake in epididymal adipose tissue of the rat is noticeably inhibited by a previous injection of cortisone acetate. MUNCK[7] and MUNCK AND KORITZ[8] have demonstrated, *in vivo* and *in vitro*, that cortisol, corticosterone and deoxycorticosterone reduce the glucose uptake of epididymal adipose tissue. In the light of our experiments with adipose tissue from adrenalectomized rats, we consider this interpretation to be, quite likely, correct.

ACKNOWLEDGEMENTS

We wish to thank Professor F. G. YOUNG for his advice and criticism. This study was supported by a research grant from the J. March Foundation (Ayuda 1960) and the Eli Lilly Foundation.

REFERENCES

1 M. PERLMUTTER, S. WEISENFELD AND M. MUFSON, *Endocrinology*, 50 (1952) 442.
2 J. L. R-CANDELA, J. ROVIRA AND R. R-CANDELA, *Medicina*, 2 (1954) 167.
3 J. L. R-CANDELA, J. ROVIRA AND R. R-CANDELA, *Rev. Iberica Endocrinol.*, 2 (1955) 787.
4 J. E. WHITNEY AND F. G. YOUNG, *Biochem. J.*, 66 (1957) 645.
5 J. L. R-CANDELA, I. VALLADARES AND R. R-CANDELA, *Rev. Iberica Endocrinol.*, 7 (1955) 2.
6 P. RIET-CORREA, E. MAGALHAES AND M. E. KRAHL, *Proc. Soc. Exptl. Biol. Med.*, 103 (1960) 704.
7 A. MUNCK, *Endocrinology*, 68 (1961) 178.
8 A. MUNCK AND S. KORITZ, *Biochim. Biophys. Acta*, 57 (1962) 310.