Hypoglycemic Effect of Adipose Tissue Extracts (ATE) in Rats

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SUMMARY

Intraperitoneal administration of ATE obtained from pooled epididymal fat pads produced hypoglycemia in adrenalectomized rats. Injection of extracts from rat brain, liver and muscle did not affect the blood glucose levels of the adrenalectomized animals. With high ATE concentrations, a progressive decline in blood sugar was observed and most of the animals died from hypoglycemia ten to thirteen hours after intraperitoneal injection. Identical concentration of ATE did not affect the blood glucose levels in intact rats.

In some adrenalectomized rats injected with ATE (100 mg.) a progressive bound insulin fall was detected, but no significant change in free insulin was evidenced. DIABETES 17:489-91, August, 1968.

It has been reported that extracts of adipose tissue (ATE) can activate in vitro the serum bound insulin of rats and human beings[1-2] and, more recently, that it is possible to induce hypoglycemia in vivo in adrenalectomized rats following intravenous injection.[3] This last finding suggests the possibility that ATE might increase glucose utilization by stimulating in vivo the utilization of bound insulin.

In this communication we report studies from our laboratory on the effect of ATE and extracts obtained from other tissues on the blood glucose levels of intact and adrenalectomized rats following intraperitoneal injection.

MATERIAL AND METHODS

Crude adipose tissue extracts were obtained from pooled epididymal fat pads of overnight fasting rats and purified by ethanol precipitation as described by Antoniades and Gundersen[1] and modified by Shaw and Shuey.[2] The pooled fat pads were homogenized with a Teflon pestle tissue homogenizer in 2-3 ml. cold distilled water per 1 gm. of tissue, centrifuged at 1,000 rpm at 5° C. per 15 min. The supernatant solution was chilled to freezing and cold 95 per cent ethanol was added, drop by drop, under continuous stirring to a final concentration of 60 per cent ethanol. The precipitate was removed by centrifugation at 2,000 rpm for 10 min. at —5° C. and discarded. The fluid supernatant was diluted with cold distilled water to approximately 10 per cent ethanol concentration, lyophilized and the dry powder was stored at —20° C. About 15-20 mg. of dry powder were obtained from 1 gm. of the original wet adipose tissue. The material was reconstituted with a phosphate buffer (pH 7.4) to a concentration of 20 mg./ml. and assayed for its in vitro biologic activity on isolated rat diaphragm in presence of rat serum, as described by Shaw and Shuey.[2] Preparations that exhibited in vitro activity were used for the in vivo studies in rats. Extracts from pooled rat brain, liver and muscle were obtained with the same technic.

The animals used in these in vivo studies were Wistar male rats from the Morini Laboratory, weighing 130-150 gm. kept on a standard diet (Purina chow) and fasting from twelve hours before the sacrifice. Adrenalectomized animals were injected seven to eight days after adrenalectomy.

Each rat was injected intraperitoneally with phosphate buffer alone (controls) or with the same volume of buffer containing ATE or other tissues extracts; ATE was given to intact and adrenalectomized animals in doses of 10-20 and 100 mg. for each animal in different groups, while brain, liver and muscle extracts were injected at a concentration of 100 mg. only in the adrenalectomized rats. A total of 300 animals was used in these studies.

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Blood samples were collected from the jugular vein of groups of animals before the injection or at various intervals after the injection. The animals were lightly narcotized with 50 per cent O_2-50 per cent CO_2 during blood collection from the jugular vein.

Blood glucose levels were determined with the glucose oxidase method.[4]

RESULTS

As shown in table 1, the blood glucose levels of the intact rats were not significantly affected by the injection of various concentrations of ATE. In contrast, the injection of ATE produced significant hypoglycemia in the adrenalectomized animals, the effect depending upon the ATE concentration. Brain, liver and muscle extracts did not produce significant effects on the blood glucose levels of the adrenalectomized rats; animals treated with control buffer had a mild hypoglycemia five to ten hours after the injection.

The rats treated with 20 mg. of ATE exhibited a decrease in blood glucose three hours after injection with progressive recovery at the fifth and the tenth hour; all the rats survived the experiment. The animals injected with 100 mg. ATE exhibited a progressive significant fall in the blood glucose levels up to the tenth hour and between the tenth and the thirteenth hour most of the rats died. Glucose values of less than 17 mg. per 100 ml. were found in the blood of animals where it was possible to measure the blood glucose immediately before the death.

Determinations of the blood glucose levels of smaller groups of animals at 30 and 60 min. after the injection of the three different ATE concentrations did not reveal any significant decrease of blood glucose levels.

DISCUSSION

The results shown in table 1 suggest that adipose tissue extracts can produce hypoglycemia in adrenalectomized rats when injected intraperitoneally. The hypoglycemic effect of ATE was directly related to its concentration.

The findings are similar to those reported recently by Antoniades[3] on the hypoglycemic effect of ATE following intravenous injection into adrenalectomized rats. The amount of ATE used by Antoniades was smaller, however, than that used in the present experiment. This may be accounted for by the fact that our preparation of adipose tissue extracts was not purified as opposed to

TABLE 1

The effect of intraperitoneal administration of ATE and other tissue extracts on blood glucose levels in intact and adrenalectomized rats*

Sample	Blood glucose mg. per 100 ml. (mean ± SD) Before injection*	Hours after injection* 3	5	10
Intact rats:				
Control buffer	54±8 (40)	49± 6 (8)	49±13 (17)	51±4 (8)
p		NS	NS	NS
ATE 10 mg. per rat		52± 2 (7)	54±11 (14)	53±3 (8)
p		NS	NS	NS
ATE 20 mg. per rat		51± 2 (8)	51±13 (6)	54±2 (8)
p		NS	NS	NS
ATE 100 mg. per rat		49± 3 (5)	52± 4 (5)	48±2 (5)
p		NS	NS	NS
Adrenalectomized rats:				
Control buffer	47±6 (12)	46±10 (9)	39± 9 (13)	40±4 (6)
p		NS	< 0.02	< 0.02
ATE 10 mg. per rat		45± 3 (8)	44± 7 (6)	40±4 (8)
p		NS	NS	NS
ATE 20 mg. per rat		25± 8 (5)	37± 7 (13)	40±5 (4)
p		< 0.001	< 0.001	NS
ATE 100 mg. per rat		28± 4 (8)	27± 5 (9)	<15 (10)
p		< 0.001	< 0.001	< 0.001
Liver extract 100 mg. per rat		46± 3 (8)	45± 2 (5)	42±3 (6)
p		NS	NS	NS
Brain extract 100 mg. per rat		45± 3 (8)	48± 3 (5)	45±3 (6)
p		NS	NS	NS
Muscle extract 100 mg. per rat		46± 5 (4)	50± 6 (4)	48±4 (4)
p		NS	NS	NS

*Number of animals in parenthesis. NS = not significant ($p > 0.05$).

those used by the author. Activity with ATE after intraperitoneal injection suggests peritoneal absorption of an active principle contained in the adipose extract with molecular weight (below 600) compatible with the active factor.[3]

Extracts obtained from brain, liver and muscle did not produce the hypoglycemic effect shown by ATE, while the slight decrease in blood glucose of adrenalectomized rats treated with buffer alone may be caused by prolonged fasting.

It is unlikely that the effects with ATE were due to the presence of insulin in the preparations: ATE alone was shown to be inactive in isolated hemidiaphragm and it retained its effects after boiling for five minutes and ultrafiltration through a filter which allowed the passage of substances of a molecular weight of less than 600.[3] A lack of activity in our muscle extracts (table 1) provides additional evidence that the activity of ATE is not due to the presence of insulin in the tissue extracts. However, direct experimental evidence on the mode of action of ATE in vivo is lacking. Several possibilities such as activation of bound insulin, pancreatic stimulation or inhibition of glucose release from hepatic stores or inhibition of gluconeogenesis may be considered.

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REFERENCES

1 Antoniades, H. N., and Gundersen, K.: Studies on the state of insulin in blood: materials and methods for the estimation of "free" and "bound" insulin-like activity in serum. Endocrinology 70:95, 1962.

2 Shaw, W. N., and Shuey, E. W.: The presence of two forms of insulin in normal human serum. Biochemistry 2:286, 1963.

3 Antoniades, H. N.: Hypoglycemic effect of adipose-tissue extracts in adrenalectomized rats. Lancet 1:602, 1967.

4 Introzzi, P.: *In* Trattato It. Med. Int.: Tecniche e Diagnostica di Laboratorio. Roma, Abruzzini. 1960, p. 80.