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ÉDITIONS DE L'ACADÉMIE DE LA RÉPUBLIQUE SOCIALISTE DE ROUMANIE

CONTRIBUTIONS TO THE STUDY OF THE MECHANISM OF AET ACTION ON THE CARBOHYDRATE METABOLISM IN WHITE RATS

BY

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The effect of AET was studied upon some aspects of carbohydrate metabolism in white rats. In normal rats, AET induced hypoglycaemia, decreased liver glycogen content and glucose-6-phosphate activity. These effects of AET were abolished by previous administration of Redergam. Atropine suppressed only the hypoglycaemic effect of AET. In alloxan-diabetic animals AET provoked hyperglycaemia and a decrease of liver glycogen level, meanwhile in the adrenalectomized rats, AET caused a decrease of the liver glycogen level only.

The theory of the biochemical shock, elaborated by Bacq and co-workers [1], attributes a particular importance to the action of radioprotective substances upon carbohydrate metabolism.

It was demonstrated by the majority of authors, that AET (S-2 aminoethyl-isothyourea Br. HBr) induces significant changes in glycaemia, in lactate and pyruvate concentration of the blood, as well as in the glycogen content of the liver [1—3], [13], [15]. It is presumed, that AET, beside its direct action on the carbohydrate metabolism, may have also any indirect effects, similarly to many radioprotective substances, structurally related with AET [1], [2]. Any of these indirect actions are interpreted by the effects of AET upon some endocrine glands important in carbohydrate metabolism. In this respect the data are contradictory [1], [3].

The object of this study is to clarify the effect of AET (administered in optimal radioprotective dosis) upon some aspects of carbohydrate metabolism in normal white rats, in rats treated with sympathicolytical substances, in alloxan-diabetic rats and in adrenalectomized rats. In this connection the following parameters were studied: glycaemia, blood lactate and pyruvate concentration, hepatic glycogen content, the acti-

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vity of glucose-6-phosphatase and hexokinase of the liver, the uptake of ^{32}P by the liver, pancreas and adrenal glands, as well as insulin-like activity of the plasma.

MATERIAL AND METHODS

Albino male rats weighing 130—160 g (mixed strain) were used. They were fed on a standard diet during 10 days, and fasted 18 hours before the experiment.

The animals were distributed in 8 experimental groups:

Group I: control rats, injected intraperitoneally with 0.5 ml isotonic NaCl solution;

Group II: animals injected intraperitoneally with a small unique dose of 30 mg AET/100 g body weight;

Group III: injected intraperitoneally with Redergam plus AET. A dose of Redergam (0.1 mg. per 100 g b.w) was injected 20 minutes before the injection of AET, and the other dose of Redergam was administered simultaneously with AET (30 mg/100 g b.w);

Group IV: injected intraperitoneally with 0.05 mg. atropine per 100 g b.w., 20 minutes before the administration of AET;

Group V: control alloxan-diabetic rats. Experimental diabetes was induced through a rapid intravenous injection of 7.5. mg alloxan/100 g b.w. Alloxan was dissolved freshly in isotonic NaCl solution, and administered subcutaneously in 18-hour fasted animals;

Group VI: alloxan-diabetic rats, injected intraperitoneally with 30 mg AET/100 g b.w 48 hours after alloxanization;

Group VII: control adrenalectomized animals, treated substitutively during 2 days with a daily dose of subcutaneously administered 2.5 mg hydrocortisone per 100 g b.w;

Group VIII: adrenalectomized rats, treated subcutaneously, with hydrocortisone (similar to group VII), injected intraperitoneally with 30 mg AET per 100 g b.w.

Blood samples for determination of initial glycaemia were collected from the tail veins, and for the determination of circulating blood glucose, blood lactate and blood pyruvate concentration, blood was obtained through decapitation of animals.

Glycaemia was determined photocolorimetrically, using a glucose oxidase method [8]. For the determination of liver glycogen content the method of Montgomery [9] was utilized and for blood lactate determination the metho dof Barker and Summerson [4]. Pyruvate concentration of blood was determined using the method of Rindi and Ferrari [12]. Hexokinase activity in the liver was followed according to the method of Kohn and Minski [9], and the liver glucose-6-phosphatase activity was determined after Marjorie and Swanson [7]. ^{32}P uptake by tissues was determined 20 minutes after intraperitoneal injection of AET and 0.5 μ Ci $Na_2 H_{32} PO_4$ g b.w., respectively. The specific radioactivity of tissues, obtained by radioactive measurements [14] was calculated using the following formula:

$$\text{coefficient} = \frac{\text{radioactivity of 100 mg tissue}}{\text{radioactivity of 0.1 ml plasma}}$$

Insulin-like activity of plasma was determined with isolated rat adipose tissue, using the method of Martin et al [16].

RESULTS

Data concerning the values of glycaemia and of liver glycogen content are summarized in Table 1.

One can see that in normal animals AET induces significant hypoglycaemia 20 minutes after its administration; the blood glucose level

Table 1

The values of glycaemia and of liver glycogen content after AET administration in various experimental conditions

Group	Glycaemia mg%		Diff. mg. % related to the initial values	P
	Initial	20 min. after AET		
I Control	85±6.1	87±5.9	+ 2	>0.5
II AET	92±5.4	58±4.2	−34	<0.01
III Redergam + AET	90±3.9	92±5.3	+ 2	> 0.5
IV Atropine + AET	88±3.1	91± 4.2	+ 3	> 0.5
V Alloxan diab.	345±31	352±27	+ 7	> 0.5
VI Alloxan diab. + AET	315±23	410±35	+95	<0.02
VII Adrenalectomy	87±3.4	90±2.8	+ 3	> 0.5
VIII Adrenalectomy + AET	84±2.8	89±11	+ 5	> 0.5
GROUP	Liver glycogen content mg%		Diff. compared to the control values mg%	P
I Control	2386±439		—	—
II AET	1269±102		−1117	< 0.02
III Redergam + AET	2191±116		−195	> 0.5
IV Atropine + AET	1135±84		−1251	<0.05
V Alloxan diab.	1015±120		—	—
VI Alloxan diab. + AET	430± 53		— 585	< 0.01
VII Adrenalectomy	3254±117		—	—
VIII Adrenalectomy + AET	2436±233		— 818	< 0.02

decreases with 34 mg%, comparative to the initial values ($p<0.01$). Simultaneously with the decrease of glycaemia, liver glycogen content decreases with 1117 mg% as compared to the control values ($p<0.02$).

In conditions of previous administration of Redergam, the blood glucose level does not change in comparison to the initial value of glycaemia ($p>0.5$), as the hypoglycaemic effect of AET is inhibited. On the contrary, Redergam reduces the glycogenolytic effect of AET, the amount of liver glycogen being decreased only with 195 mg% as compared to the control values ($p>0.5$). The liver glycogen content of this group increases significantly in comparison with the liver glycogen content of rats, treated only with AET (+922 mg%; $p<0.05$).

In normal rats, which were injected with atropine, glycaemia is not modified 20 minutes after AET administration. In this case the glycogen-mobilizing effect of AET is not influenced by atropine.

In alloxan-diabetic animals, AET induces a significant increase of glycaemia (+95 mg%; $p<0.02$), and a marked decrease of liver glycogen content (—585 mg%) as compared to the alloxan-diabetic control animals ($p<0.01$).

In adrenalectomized and substitutively hydrocortisone-treated rats AET does not show any changes in glycaemia as compared to the initial values and to the values of control adrenalectomized animals, respectively. On the contrary, the liver glycogen content of this group decreases with 818 mg%, related to the liver glycogen content of control adrenalectomized rats ($p<0.02$).

Table 2 show the values of blood lactate and pyruvate concentrations.

Table 2

Action of AET on blood lactate (AL) and blood pyruvate (AP) concentration

Group	Lactate mg%	P	Pyruvate mg%	P	AL/AP
Control	11.69±0.3	—	1.21±0.17	—	9.7
AET	28.1±2.8	>0.01	2.29±0.30	>0.01	12.2

From these data it results that 20 minutes after AET injection, blood lactate concentration increases from 11.69 mg% to 28.10 mg% ($p>0.01$), and pyruvate concentration from 1.21 mg% to 2.29 mg% ($p<0.01$).

The values of liver hexokinase and glucose-6-phosphatase activity, as well as the insulin-like activity of plasma are summarized in Table 3.

These data show that AET stimulates significantly the activity of liver hexokinase ($p<0.01$), and inhibits glucoso-6-phosphatase activity ($p<0.02$). Simultaneously with modification of above parameters, one can observe the increase of plasma insulin-like activity ($p<0.05$).

The action of AET on the ^{32}P uptake in the adrenal and pancreatic glands and in the liver tissue is shown in table 4.

It is demonstrated that AET increases the ^{32}P uptake in the adrenal and pancreatic glands, as in the liver, meaning a hyperactivity of all these tissues.

Table 3

Effect of AET on liver hexokinase and glucoso-6-phosphatase activity, and on the insulin-like activity of plasma (ILA)

Group	Hexokinase mg. gluc./100 mg protein	Glucoso-6-phosphatase mg P_i/100 mg protein	I.L.A. μU/m
Control n = 10	X = 0.064 ES = 0.017	X = 0.768 ES = 0.033	185 ±19
AET n = 10	X = 0.739 ES = 0.096 p < 0.01	X = 0.421 ES = 0.06 p < 0.02	343 ±42 p<0.05

Table 4

Distribution of P^{32} in various organs by control and AET treated groups

Groups	cpm 100 mg adrenal gl. cpm 0.1 ml plasma	cpm 100 mg pancreas cpm 0.1 ml plasma	cpm 100 mgr liver cpm 0.1 ml plasma
Control n = 10	X = 3.72 ES = 0.37	X = 4.15 ES = 1.27	X = 9.73 ES = 1.5
AET n = 10	X = 4.48 ES = 0.30 p > 0.1	X = 7.80 ES = 1.69 p>0.1	X = 12.7 ES = 1.71 p = 0.2

DISCUSSION

Our experimental data evince that AET induces a significant decrease of glycaemia and of liver glycogen amount. These data are in agreement with the observations of Zins et al. [15], who obtained similar modifications in glycaemia and in liver-glycogen content after AET administration.

The above modifications in carbohydrate metabolism are associated with the increase of blood lactate and pyruvate concentration, as well as with the stimulation of liver hexokinase activity and inhibition of liver glucose-6-phosphatase activity.

The mechanism by which AET induces modifications in carbohydrate metabolism are likely complex mechanisms. From our results it arises that AET manifests an adrenal-independent direct stimulative effect upon liver hexokinase activity, demonstrated by the data obtained in adrenalectomized rats (group VII and VIII). The direct action of this substance on liver glucose-6-phosphatase activity may be interpreted also by the absence of hyperglycaemia. On the other hand, Pora et al. demonstrated

that by a direct mechanism AET induces a partial inhibition in the activity of tissue respiratory enzymes [10], which leads to the increase of anaerobic glycolysis, accompanied with the increase of blood lactate and pyruvate concentration.

As for the indirect action of AET upon the carbohydrate metabolism, our data showed that the effect of this radioprotective substance may be manifested by a few effects upon some endocrine glands. The fact that by previous administration of Redergam hyperglycaemic and glycogenolytic action of AET is annihilated demonstrates the intervention of adrenaline in this fenomenon, and that the secretion of adrenaline is probably stimulated by AET treatment.

At the same time, our results showed that AET acts upon the secretory activity of endocrine pancreas, increasing the insulin-like activity of the plasma. This result is in concordance with our above experimental data, which demonstrate that the insulin secretion after rapid intravenous hyperglycaemia stimulus may be inhibited by previous administration of atropine [6]. In fact, the present experiments demonstrate that atropine inhibits the hypoglycaemic effect of AET. On the other hand, the data obtained in alloxan-diabetic animals demonstrate that, in absence of circulating insulin, AET manifests a significant hyperglycaemic effect, which is associated with increased glycolysis. In these circumstances, the influence of adrenal glands becomes preponderent.

CONCLUSIONS

1. The hypoglycaemic and glycogenolytic effect of AET is achieved through a complex direct and indirect mechanism.
2. The direct action of AET is manifested in increase of liver hexokinase activity and in diminution of liver glucose-6-phosphatase activity.
3. The indirect action of AET on the carbohydrate metabolism in white rats is achieved by modification in the activity of some endocrine glands: for instance the adrenal glands and the endocrine pancreas.

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