THE EFFECT OF ADRENALINE INJECTION IN THE UTILIZATION OF GLUCOSE IN MUSCLE EXTRACTS

by

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INTRODUCTION

In a previous paper (COHEN[1]) experiments were described, showing the decrease, after adrenaline injection, in muscle glycolysis due to glucose as substrate. In that work, pieces of diaphragm muscle were used. It was shown (COHEN[2]) that the adrenaline inhibition was probably an indirect effect, mediated by the hypophysis but not requiring the presence of the adrenal glands. The work to be reported in the present paper was done in order to see whether the diminished glucose utilization made itself seen also in muscle extracts from adrenaline injected animals and if so whether any information could be obtained about the enzyme mechanisms concerned in the inhibition.

EXPERIMENTAL METHODS

The rats used were of the same breed (piebald Norwegian) and the same weight (100–120 g) as those of the previous paper (COHEN[1]). They were fasted for 19–24 hours before the experiment. Adrenaline was injected subcutaneously (dose, 1 mg/kg) 50–70 minutes before the death of the animals. The solution was made by dissolving the base in a little 0.05 *N* HCl; the volume was then made up with water (final acid concentration, about 0.01 *N*). The injected animals and the controls were killed by decapitation and, as soon as bleeding ceased, the muscles of the hind limbs were rapidly removed and cooled in ice. The further preparation was done in a room at 0° C. The muscles were finely chopped, then ground with 1½ times their weight of icecold water, either in a glass homogeniser or in a mortar. After standing 5 minutes, the mass was strained through fine handkerchief cotton, and the filtrates were used at once for the measurements of glucose consumption.

The experiments were carried out at 30° C and at p_H 7.5; the medium used was 0.2% $NaHCO_3$ with an atmosphere of 95% N_2/5% CO_2 above. For ease of manipulation, the pots attached to Barcroft manometers were used. The various additions made are detailed in the tables. The experiments consist of four series:

1a. Those of short duration—12 to 15 minutes—in the presence of *M*/16 fluoride. Since a high initial concentration of ATP was provided, and its dephosphorylation by ATPase was inhibited by the fluoride, the ATP concentration does not become a limiting factor in the period of the experiment under these conditions and the disappearance of glucose is a measure of the hexokinase activity.

1b. Those of short duration in the absence of fluoride.

2a. Those of longer duration (35 minutes) in the absence of fluoride. In these, co-enzyme I was added as well as ATP and the continued glycolysis no doubt depends not only on the initial concentration of ATP but on its continual resynthesis from ADP by means of the diphosphoglyceric and phosphopyruvic acids formed during glycolysis.

2b. Those of longer duration in the presence of fluoride.

Since any changes in enzyme activity consequent upon the adrenaline action might be of a transient or unstable nature, it seemed best to mix all the components of the experiment with as little delay as possible. All the additions except the muscle extract, were placed in the Barcroft pots; then the appropriate extract was introduced into each and a sample was withdrawn. The pots were immediately fixed to the manometers and put into the bath where gassing took place. By careful

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timing, the treatment of the samples from the moment of adding the extract was made exactly similar in all cases. At the end of the period, a second sample was removed.

Each sample (1 ml) was run into 3 ml water. 0.75 ml 0.3 *N* $Ba(OH)_2$ was added, and then 0.75 ml 5% $ZnSO_4.6H_2O$. The deproteinisation and estimation of glucose in the filtrate followed, according to NELSON[3].

The estimation of phosphates was by the method of FISKE and SUBARROW[4].

The ATP was made by the method of LOHMANN as described by NEEDHAM[5] and the co-enzyme I by the method of WILLIAMSON AND GREEN[6].

EXPERIMENTAL RESULTS

The results for glucose utilization are summarized in Tables I and II.

TABLE I

THE GLUCOSE DISAPPEARING IN MUSCLE EXTRACTS FROM NORMAL AND ADRENALINE-INJECTED RATS

Experimental period, 12 minutes at 30°

ATP (mg 9'P) per sample	a. With NaF present; 0.063 *M*		b. In absence of NaF	
	Normal	Injected	Normal	Injected
1.05	0.56	0.55	0.65	0.52
1.05	0.63	0.34	0.69	0.44
1.05	0.59	0.61	0.72	0.67
1.05	0.43	0.52	0.69	0.64
1.05	0.40	0.41	0.71	0.56
1.05	0.54	0.50	0.75	0.65
1.05	0.39	0.60	0.70	0.66
1.07	0.77	0.69		
0.79 } 0.66 } 0.53	0.59 } 0.58 } 0.36	0.56 } 0.61		
0.81 } 0.45	0.83 } 0.41	0.53 } 0.37		
0.5	0.36	0.42	0.41	0.44
	Av: 0.53 ± 0.038*	Av: 0.52 ± 0.031*	Av: 0.67 ± 0.038*	Av: 0.57 ± 0.034*

1 ml muscle extract was used in a final volume of 3 ml. The concentrations of other constituents were: $NaHCO_3$, 0.2%; glucose, 0.05%; $MgCl_2$, 0.005 *M*; phosphate buffer, p_H 7.5, 0.02 *M*. Atmosphere: 95% N_2, 5% CO_2.

The figures give mg glucose disappearing per sample. The results appearing on the same horizontal line were obtained for normal and injected respectively on the same extract. Two rats were used for each extract in the table above. Where results are bracketed together these also were on the same extract.

* Standard Error of the Mean ($T/\sqrt{N}$).

Test for significance of adrenaline injection (FISHER[7]).

Group a: $t = 0.204$; $p = > 0.8$

b: $t = 1.958$; $p = 0.05$

It will be seen that, in the presence of fluoride, there is no significant difference between the values for extracts from the normal and injected animals. In the absence of fluoride, in the experiments of longer duration, there is a marked decrease in the power to use glucose in the extracts from injected animals. This decrease is noticeable though considerably smaller, in the experiments of only 12 minutes duration.

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TABLE II

THE GLUCOSE DISAPPEARING IN MUSCLE EXTRACTS FROM NORMAL AND ADRENALINE-INJECTED ANIMALS

Experimental period, 35 minutes at 30°

ATP (mg 9'P) per sample	a. In absence of NaF		b. With NaF present; 0.063 *M*	
	Normal	Injected	Normal	Injected
1.0	1.90	2.04	1.11	1.23
1.0	2.06	1.44	1.06	1.01
0.8	1.69	2.05	0.86	1.05
0.8	1.22	1.90	0.75	0.78
0.8	2.01	0.85	0.91	0.97
0.8	2.00	1.52	0.93	1.15
0.64	0.94	1.99	0.89	1.05
0.94	0.88	0.34	0.80	0.80
0.94	1.23	0.64	1.11	1.38
0.94	1.87	0.97	1.03	1.18
0.96	0.64	0.19	0.41	0.68
0.96	1.35	0.45	0.74	0.81
0.96	1.93	1.45	0.53	0.41
0.98	0.89	0.52		
0.98	1.39	0.66		
	Av: 1.40 ± 0.127*	Av: 0.96 ± 0.129*	Av: 0.84 ± 0.059*	Av: 0.93 ± 0.073*

0.6 ml muscle extract was used in a final volume of 2 ml. The concentrations of other constituents were: $NaHCO_3$, 0.2%; glucose 0.2%; $MgCl_2$, 0.005 *M*; phosphate buffer, pH 7.5, 0.02 *M*; co-enzyme I, 0.00016 *M*; Atmosphere: 95% N, 5% CO_2.

The figures give mg glucose disappearing per sample. The results appearing on the same horizontal line were obtained, for normal and injected respectively, on the same extract. One rat was used for the preparation of each extract in the first 7 experiments, two rats for each in the later experiments; so that 25 normal and injected were used in the series without fluoride, and 21 of each in the series with fluoride.

* Standard Error of the Mean ($T/\sqrt{N}$).
Test for significance (FISHER[7]).
Group a: $t = 2.431$; $P = 0.02$
b: $t = 0.958$; $P = 0.4$

TABLE III

THE GLUCOSE DISAPPEARANCE IN MUSCLE EXTRACTS FROM NORMAL AND ADRENALINE-INJECTED ANIMALS, WITHOUT FLUORIDE

Experimental period, 20 and 35 minutes

Time	Normal	Injected	Inhibition in injected
20'	0.52	0.33	36%
35'	0.89	0.52	41%
20'	1.09	0.65	41%
35'	1.39	0.66	53%

The conditions were the same as in Table IIa, ATP added: 0.98 mg 9' P per sample. 2 rats for each extract.

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Two experiments, shown in Table III, illustrate the rate at which the difference in behaviour between the normal extracts and those from treated animals becomes apparent.

No systematic study was made of the phosphate fractions during the glucose utilization under these different conditions; but some estimations were made and a few results will be given here to indicate the magnitude of the changes going on and in particular the part played by ATPase.

TABLE IV

	Time	P_9	P_0	P_9–P_0	Glucose used: Calc. from P_9–P_0 disappearing	Glucose used: estimated
1. No Glucose added	0 30′	3.10 3.10	1.62 2.30	1.48 0.8		
2. Glucose added	0 35′	3.10 2.22	1.62 1.62	1.48 0.6	2.64	2.46

The figures give mg per sample.

Table IV shows the changes in P_0 and P_9 for an experiment with normal extract carried out under the same conditions as in Table IIb (i.e. with fluoride present). A sample to which no glucose was added is included.

TABLE V

Time	Normal, no F	Inj., no F	Normal, with F	Inj., with F
0	2.04	1.93	1.83	1.90
10	2.71	2.60	1.76	2.15
30	3.11	3.04	2.10	2.19
45	3.18	3.06	2.02	2.11

The figures give mg inorganic P per sample.

Table V shows the course followed by the inorganic P content for extracts from normal and adrenaline-injected animals, in an experiment of the type of Table II.

TABLE VI

Time	Normal	Injected
0	0.67	0.67
1	1.25	1.25
5	1.65	1.91
15	3.00	3.30
30	4.32	4.35

0.3 ml extract was used in a final volume of 3 ml. The concentrations of other constituents were: ATP, 1.15 mg 9′ P per sample; veronal buffer, 0.04 *M*; $MgCl_2$, 0.005 *M*. The figures give mg inorganic P per ml muscle extract.

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Finally, Table VI gives the results of an experiment designed to find the rate of ATP dephosphorylation by ATPase in the absence of glucose. Samples (0.3 ml) of extract were incubated at 30° C with ATP in veronal buffer at PH 7.5.

DISCUSSION

The experiments described show that there is no difference between extracts of muscles from normal and adrenaline injected animals as regards glucose consumption, when this is measured in presence of an excess of ATP. This indicates that the hexokinase activity in the extracts from the injected animals is unimpaired*. On the other hand, when the glucose uptake is measured over a longer period and in absence of fluoride, the extracts from injected animals show markedly less power to use glucose. Under these conditions, the ATPase of the extracts is paying a large part, as may be seen if the rate of inorganic phosphate formation in experiment 1; Table IV is compared with the rate in Table VI. The rate of rephosphorylation of ADP, by means of the diphosphoglycerate and phosphopyruvate formed during glycolysis thus becomes important, and it seems likely that it is in some step of this resynthesis that the extracts from the injected animals are defective.

We have at present no information which would enable us to specify at what stage the loss of activity occurs. Presumably the inhibition of any stage beyond the point at which it became the limiting factor could lead to diminished rate of glycolysis and so of resynthesis of ATP. Increased ATPase activity would, of course, have the same effect as diminished resynthesis of ATP; the few experiments we have done comparing the ATPase activity of the two types of extract showed no significant difference, but this possibility should be further explored.

With regard to the experiments of longer duration in the presence of fluoride (series 2b) Tables IV and V indicate that here too to a less extent resynthesis of ATP is going on at the expense of inorganic P (*via* diphosphoglycerate). That no difference in glucose uptake is seen in the two types of extract under these conditions cannot however be taken to mean that the loss of activity consequent on adrenaline injection must occur at some stage after phosphoglycerate formation. In the presence of fluoride, the continued oxidation of glyceraldehyde phosphate by co-enzyme depends on the oxidation of the reduced co-enzyme by other molecules of glyceraldehyde phosphate, themselves reduced to glycerol phosphate. The glycerol phosphate dehydrogenase concerned here is usually less active than the lactic dehydrogenase concerned when the reduced co-enzyme is oxidised by pyruvate (see MEYERHOF AND KIESSLING[9]); this is probably the explanation of the greatly increased glucose utilization in series 2a, without fluoride as compared to series 2b, with fluoride. Since, therefore, in the presence of fluoride, resynthesis of ATP is going on at a comparatively low rate on account of the glycerol phosphate dehydrogenase involved, it is likely that considerable depression of the activity of another enzyme in the system might take place without its becoming the limiting factor.

* In a preliminary publication (COHEN AND NEEDHAM[8]) it was stated that the hexokinase activity shows an inhibition of about 13% after adrenaline injection. The series of results upon which this statement depended were all obtained using one sample of ATP. Since this inhibitory effect was not found again in later experiments, in which a number of different preparations of ATP were used; we are of opinion that it was probably due to deterioration of the ATP sample, so that less 9'P was being added than we supposed. Co-enzyme I was added in this first series, and the conditions for resynthesis were thus good.

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SUMMARY

1. The uptake of glucose by extracts made from skeletal muscles of rats 50 to 60 minutes after subcutaneous injection of adrenaline (1 mg/kg) was investigated.

2. When conditions were such that ATP resynthesis became important (experiments lasting half an hour or more in the absence of fluoride) the glucose utilization was markedly diminished.

3. Under conditions where the original concentration of ATP was well maintained (short duration-experiments with addition of fluoride to inhibit ATP-ase) no diminution was observed.

4. These facts seem to indicate that hexokinase is not affected but that there is a decreased rate of ATP resynthesis in the extracts obtained from animals treated with adrenaline.

RÉSUMÉ

1. Nous avons étudié l'absorption de glucose par des extraits préparés à partir de muscles du squelette de rats 50 à 60 minutes après injection souscutanée d'adrénaline (1 mg/kg).

2. Dans des conditions où la régénération d'ATP devenait importante (expériences d'une demi-heure ou plus en absence de fluorure) l'utilisation de glucose était diminuée de façon notable.

3. Sous des conditions où la concentration initiale d'ATP était maintenue constante (expériences de courte durée, addition de fluorure pour inhiber l'ATP-ase) nous n'avons pas observé de diminution.

4. Ces faits semblent indiquer que l'hexokinase n'est pas affecté mais que la régénération d'ATP est ralentie dans les extraits préparés à partir d'animaux ayant subi un traitement préalable d'adrénaline.

ZUSAMMENFASSUNG

1. Die Aufnahme von Glucose wurde in Skelettmuskel-Extrakten von Ratten untersucht, welche 50 bis 60 Minuten vorher eine subkutane Adrenalininjektion (1 mg/kg) erhalten hatten.

2. Unter Bedingungen, wo die ATP-Erneuerung bedeutend wurde (Versuche von einer halben Stunde oder mehr in Abwesenheit von Fluorid) war der Verbrauch an Glucose bedeutend vermindert.

3. Unter Bedingungen wo die ursprüngliche ATP-Konzentration erhalten blieb (kurze Versuchsdauer, Zugabe von Fluorid zur Hemmung der ATP-ase) wurde keine Verminderung beobachtet.

4. Diese Tatsachen scheinen darauf hinzuweisen, dass in Extrakten aus mit Adrenalin vorbehandelten Tieren die Hexokinase nicht angegriffen, die Geschwindigkeiten der ATP-Erneuerung aber verlangsamt ist.

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