An Action of Adenosine 3′,5′-Monophosphate on Glucose Metabolism of Rabbit Brain Slices

Adenosine 3′,5′-monophosphate (AMP) is known to activate phosphorylase[1], which activates glycogenolysis[2]. AMP also activates lipase, thus inducing a lipolysis[3]. These mechanisms cause a transient hyperglycemia[4] and a longer lasting reduction of fatty acids in the plasma[5]. In vitro, AMP was found to inhibit the growth of HeLa and strain L cells in culture[6].

Since the brain is the mammalian tissue with the highest ability to synthesize AMP[7], we have studied the influence of AMP on the glucose metabolism of rabbit brain slices.

Method. The detailed method is described in a previous paper[8]. Slices from the rabbit forebrain of a thickness of less than 0.3 mm were prepared at a temperature near 0 °C. The metabolism was measured using Warburg's[9] new manometric two-vessel method. The temperature was 38 °C, the atmosphere within the vessels consisted of N_2–O_2–CO_2 (75–20–5), the CO_2 partial pressure was kept constant over the period of the experiment. The first manometer readings were taken between 95 and 105 min after the death of the animals. The composition of the incubation medius was: NaCl (106 m*M*), KCl (4.7 m*M*), $CaCl_2$ (2.5 m*M*), $MgSO_4$ (0.8 m*M*), KH_2PO_4 (0.3 m*M*), Na_2HPO_4 (1.2 m*M*), $NaHCO_3$ (25 m*M*), and glucose (2 mg/ml, 1 mg/ml, 0.5 mg/ml, respectively). As a control, the production of lactic acid and the consumption of glucose were determined by analysis of the incubation solutions with the specific lactate dehydrogenase and hexokinase/glucose-6-phosphate dehydrogenase optical methods.

Results and discussion. As presented in the Table (a) and (b), the influence of AMP on the glucose metabolism of rabbit brain slices was investigated by measuring the aerobic glycolysis and respiration in vitro. The influence of AMP on the glucose metabolism depends upon the concentration of AMP within the incubation medium.

(1) 0.5–1.0 m*M* AMP (experiments 1, 2, 3 and 5, presented in the Table). During the first 30 min after the addition of AMP the aerobic glycolysis increased strongly and the respiration increased slightly. Later on the glycolysis completely subsided, whereas the respiration was further stimulated.

(2) 0.1 m*M* AMP (experiments 4 and 5). Immediately after the addition of AMP no increase of the aerobic glycolysis could be found (perhaps the stimulation period is too short to be observed by a conventional manometric method). Towards the end of the experiment an inhibition of the glycolysis took place. In one experiment this inhibition was complete, in another it was only partial. The respiration was always enhanced.

(3) 0.01 m*M* AMP (experiment 5). At this concentration there only seemed to be a slight stimulation of the respiration. The glycolysis was not influenced.

Figures 1 (a and b) and 2 (a and b) demonstrate the influence of 1.0 m*M* and 0.1 m*M* AMP on the in vitro metabolism of rabbit brain slices. The time course of the action of AMP is not exactly the same in all experiments, but the effects are well reproducible. An explanation of the effects is not yet possible, but our observations explain the inhibitory action of AMP on cell growth in vitro: it is the inhibition of aerobic glycolysis that inhibits cell multiplication via a deficit of energy. This explanation is supported by the good agreement between the minimum concentrations necessary for inhibition: the least concentration required for an inhibition of cell growth is 0.12 m*M* AMP per litre and the minimum concentration for the inhibition of the glycolysis of rabbit brain slices is also near 0.1 m*M* AMP per litre.

After completion of our experiments an interesting biochemical action of AMP was reported by MIYAMOTO,

The influence of adenosine 3′,5′-monophosphate (AMP) on aerobic glycolysis and respiration of rabbit-brain slices

(a) Glycolysis. Q_M [μl CO_2 from glycolysis/mg dry weight/h][a]

Experiment	Before addition	After addition 0–30 min	30–70 min	70–120 min
(1) Control	+2.6	+1.6	+1.4	+2.1
1.0 m*M* AMP		+8.0	+0.3	0.2
(2) Control	+3.1	+2.4	+1.8	+2.3
1.0 m*M* AMP		+8.6	+2.4	+0.5
(3) Control	+2.2	+2.0	+2.7	+2.5
0.5 m*M* AMP		+9.5	+0.8	−0.3
(4) Control	+1.7	+2.2	+0.8	+1.1
0.1 m*M* AMP		+1.4	+0.5	+0.3
(5) Control	+3.9	+3.4	+3.5	+3.1
0.01 m*M* AMP		+3.0	+3.3	+3.0
0.1 m*M* AMP		+2.4	+1.8	+2.2
1.0 m*M* AMP		+7.8	+1.2	+0.4

(b) Respiration. QO_2 [μl O_2/mg dry weight/h]

(1) Control	−4.7	−3.9	−4.1	−3.6
1.0 m*M* AMP		−3.8	−5.5	−5.3
(2) Control	−3.8	−3.2	−5.3	−4.1
1.0 m*M* AMP		−4.0	−6.4	−6.4
(3) Control	−4.4	−3.2	−2.9	−4.3
0.5 m*M* AMP		−4.0	−6.4	−5.6
(4) Control	−4.7	−3.9	−4.3	−4.2
0.1 m*M* AMP		−4.4	−4.2	−4.3
(5) Control	−5.0	−3.8	−4.2	−4.0
0.01 m*M* AMP		−4.8	−4.5	−4.1
0.1 m*M* AMP		−5.4	−5.1	−4.8
1.0 m*M* AMP		−4.4	−6.0	−4.7

[a] Calculated from the total CO_2 and the O_2 amounts with the assumption of a respiratory quotient of 1.0. For experimental conditions see text.

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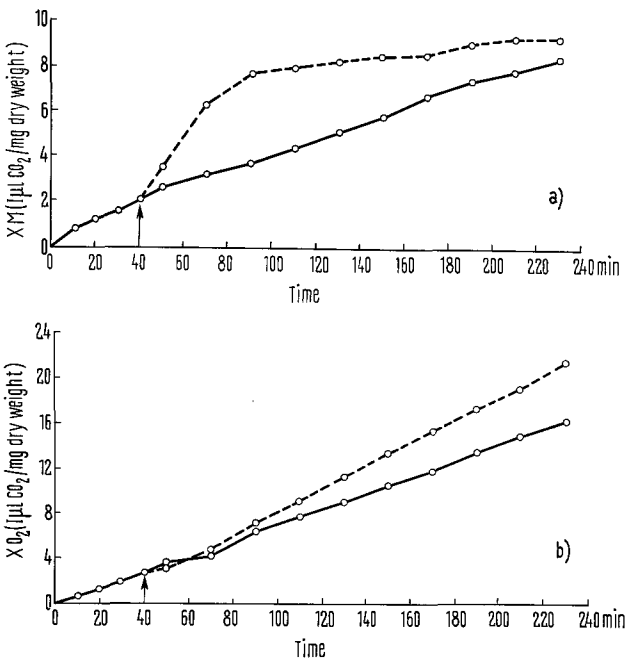

Fig. 1. Time course of (a) aerobic glycolysis and (b) respiration of rabbit-brain slices. ↑, addition of adenosine 3′,5′-monophosphate (AMP), final concentration 1 m*M*; ○—○, control; ○- - -○, presence of AMP.

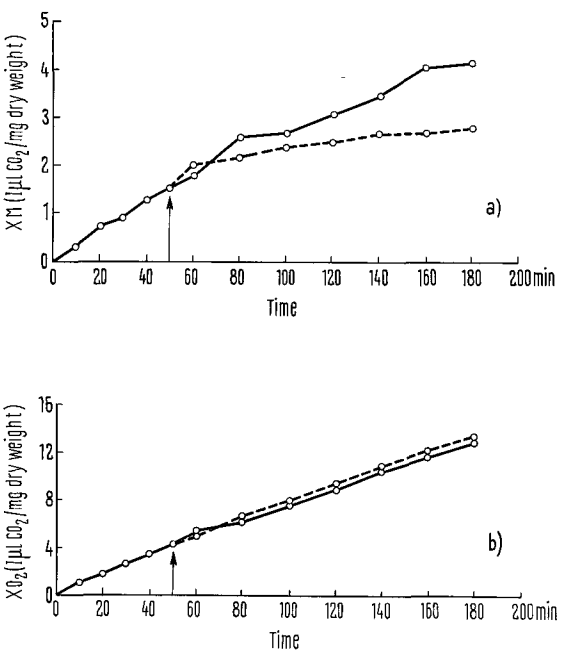

Fig. 2. Time course of (a) aerobic glycolysis and (b) respiration of rabbit-brain slices in the presence of 0.1 m*M* AMP. For symbols see Figure 1.

KUO, and GREENGARD[10]. They found that brain protein kinase was still stimulated by a concentration of 0.0005 m*M* AMP per litre incubation solution.

Zusammenfassung. Der Einfluss von Adenosin-3′,5′-monophosphat (AMP) auf den Glukosestoffwechsel von Grosshirnrindenschnitten des Kaninchens wurde untersucht. 0.1 bis 1 m*M* AMP pro Liter Incubationslösung stimulieren die Atmung der Schnitte. Die aerobe Glykolyse der Schnitte wird durch 0,5 bis 1 m*M* AMP anfänglich stark gesteigert und nach ca. 30 min völlig gehemmt. 0.1 m*M* AMP hemmt die Glykolyse lediglich.

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The Properties of Neuraminidase-Treated Crystalline Ceruloplasmin

Although the glycoprotein nature of ceruloplasmin has been described[1,2], little is known of the significance of the carbohydrate moiety for the function of this copper-containing protein. In a recent report by MORELL et al.[3], it has been shown that sialic-acid residues of ceruloplasmin protect the protein from being rapidly incorporated into the liver. In the present investigation the importance of sialic-acid residues of ceruloplasmin for its oxidase activity, physical properties and inhibition of viral hemagglutination has been studied.

Ceruloplasmin was obtained in a partially purified form from AB Kabi, Stockholm (retroplacental source) and recrystallized 3 times according to the method of DEUTSCH[4]. It had absorbance ratio ($A_{280}:A_{610}$) of 23.2 and appeared as one single protein band upon electrophoresis and ultracentrifugation. Liberation of sialic-acid residues was obtained by treatment of ceruloplasmin with neuraminidase either from *Cl. perfringens* (Type V enzyme from Sigma) or from *V. cholerae* (General Biochemicals). Sialic acid was determined by the thiobarbiturate method of WARREN[5]. The total sialic acid determined by hydrolysis in 0.1 *N* H_2SO_4 (Figure 1) amounted to 13 moles per mole ceruloplasmin (according to M.W. of 160,000[6]) while 9 moles sialic acid per mole cruloplasmin was removed enzymatically by treatment with neuraminidase. This value for sialic acid as well as the content of glucosamine[7] (23 moles), hexose[8] (26 moles) and fucose[9] (4 moles) in this preparation of crystalline ceruloplasmin are of the same order of magnitude as reported for ceruloplasmin

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