Z. Ernährungswiss. 18, 42–50 (1979)

ISSN 0044-264 X

National Research Centre, Dokki, Cairo (Egypt)

Effect of exogenous Adenosine Triphosphate on the oxygen uptake of skeletal muscle and liver slices and homogenate of albino rats

H. M. Tahani, M. Ibrahim Khairia, Y. A. Habib, and *M. Talaat*

With 5 tables

(Received July 25, 1978)

Adenosine Triphosphate (ATP) has a particular importance for its multiple functions. It presides in transportation and distribution of the energy transformation in the different phases of tissue metabolism.

Atkinson (1) reported that energy metabolism in general is controlled by the relative concentrations of ATP/ADT/AMP inside the cell. The increase in oxygen consumption during exercise is ultimately determined by the provision of large amounts of ADP to the muscle mitochondria through the rapid splitting of ATP during muscle contraction (14). Other investigators have also shown that low concentration of ATP in fasted rats are associated with low oxygen consumption in liver slices (16).

In experiments on whole animal we found that intravenous infusion of ATP resulted in increased metabolism of anesthetized dogs (34). However, the intramuscular administration of ATP resulted in a decreased metabolic rate of albino rats (35).

There is considerable evidence that the metabolic rate of an animal is the sum of the rates of respiration of various organs (25). Nevertheless tissue slice is thought to represent the organized surviving tissue and its metabolism at least qualitatively if not quantitatively (11).

The present work was undertaken to investigate the effect of ATP on the oxygen consumption of isolated tissues of albino rats.

Materials and methods

The effect of exogenous ATP on the oxygen uptake of liver and skeletal muscle was investigated using the Warburg Manometric technique. The oxygen consumption was expressed in $mm^3/g/hour$. The estimations were undertaken on the fresh muscle and liver of albino rats in absence and presence of ATP.

The weight of the animals ranged from 120–180 g. The animals were killed by a blow on the head, followed by cutting the throat. The liver and muscle were removed and were kept in Krebs Ringer phosphate buffer solution. Two types of liver and muscle preparations were used, namely slices and homogenate.

Experiments using the skeletal muscle or liver slices

Each diaphragm was cleaned of fat and connective tissue, and its central portion was removed. The hemidiaphragm was placed in two separate flasks. One is considered as control for the other which is exposed to exogenous ATP.

Technique for liver slicing

Liver slices 0.5 mm thick were prepared using the "Stadie Riggs" tissue slicer (Arthur H. Thomas Co., Philadelphia 5, Pa, USA).

The technique used in the preparation of the slices was according to the direction given by *Stadie* and *Riggs* (31).

Two slices from the same liver were put in two separate flasks, one acting as control for the other which was exposed to exogenous ATP. Estimation of the oxygen consumption at 37 °C was undertaken, using Krebs Ringer phosphate buffer (28) as an assay medium. After the estimation of the oxygen uptake, the slices were weighed on a torsion balance (500 mg capacity).

The effect of exogenous ATP on the oxygen uptake by the rat diaphragm and liver slices was investigated. The doses of ATP used were 0.5 mg/3 ml (0.275 mM) 1 mg/3 ml (0.55 mM); 2 mg/3 ml (1.1 mM); 5 mg/3 ml (2.75 mM) and 10 mg/3 ml (5.5 mM).

Preparation of the liver and muscle homogenate

The homogenizer used for the preparation of the liver and muscle homogenate was *Townson* and *Mercer*, Craydon (England). The speed at which this homogenizer operates is about 500 rpm.

The liver was removed from the preservation medium and weighed on a torsion balance. The weight of the tissue used ranged between 300 and 400 mg. The weighed liver tissue was dropped into the homogenizer tube containing 9 times the weight of the liver-0.25 M sucrose solution to obtain a 10% homogenate, 0.4 ml of this 10% homogenate was then suspended in 2.6 ml of the specific reaction medium (table 1) already placed in the Warburg reaction flask.

For preparation of muscle homogenate the same steps used for preparing liver homogenate were followed but water is used instead of 0.25 M sucrose since it gave better results.

Results and discussion

The results obtained in the present work have shown that exogenous ATP in concentration of 0.275, 0.55, and 1.1 mM causes no significant changes in both isolated rat diaphragm and muscle homogenate while the concentration of 0.55 mM ATP causes a stimulatory effect on the oxygen uptake in case of liver homogenate.

Also the effect of exogenous ATP on the oxygen consumption varied according to its concentration. While the lower concentrations caused a stimulatory or no effect on the oxygen uptake, higher concentrations, namely 2.75 and 5.5 mM, exhibited an inhibitory effect in both muscle and liver as slices or homogenates (tables 2, 3, 4, and 5).

Table 1. Reaction medium used for

Rat liver homogenate	Rat muscle homogenate
0.3 ml 0.1 M/KH2 PO4	0.3 ml 0.1 M KH2 PO_4
0.06 ml 0.1 M/Na succinate	0.06 ml 0.6 M Na succinate
0.06 ml 0.1 M/Na pyruvate	0.06 ml 0.1 M Na pyruvate
0.4 ml 0.03 M/Mg chloride	0.4 ml 0.03 M Mg chloride
0.3 ml 0.01 M/Na-ATP	0.3 ml 0.01 M Na-ATP
0.51 ml water	0.51 ml water
0.97 ml 0.5 M sucrose	0.57 ml 1.3 M sucrose
0.4 ml 10% homogenate	0.8 ml 10% muscle homogenate

Table 2. Effect of different concentrations of ATP on the oxygen uptake by the isolated rat diaphragm at 37 °C.

No. of expt.	Conc. of ATP	Oxygen uptake in mm^3/g wet wt diaphragm/hour 1st hour control	1st hour treated	2nd hour control	2nd hour treated
10	0.55 mM 1 mg ATP/3 ml	1172.1 ±146.0	1337.3 ±347.9 insig.	988.4 ±152.4	1131.4 ±314.2 insig.
16	1.1 mM 2 mg ATP/3 ml	1125.3 ± 98.1	1032.2 ±177.6 insig.	978.1 ±190.1	835.7 ±215.6 insig.
11	2.75 mM 5 mg ATP/3 ml	1199.4 ±103.0	786.4 ±215.5 sig. decrease $P < 0.01$	999.0 ±104.7	432.3 ±129.8 sig. decrease $P < 0.01$
13	5.5 mM 10 mg ATP/3 ml	1312.3 ±130.1	1012.1 ±164.8 sig. decrease $P < 0.01$	1135.0 ±165.1	479.6 ±133.0 sig. decrease $P < 0.01$

Table 3. Effect of different concentrations of ATP on the oxygen uptake by the fresh muscle homogenate at 37 °C.

No. of expt.	Conc. of ATP	Oxygen uptake in mm^3/g wet wt muscle homogenate/hour 1st hour control	1st hour treated	2nd hour control	2nd hour treated
13	0.275–0.55 mM 0.5–1 mg ATP/3ml	2548.13 ±263.35	2362.15 ±323.07 insig.	1247.2 ±138.2	1225.5 ±166.03 insig.
6	1.1 mM 2 mg ATP/3ml	2318.03 ±274.8	1905.5 ±388.3 insig.	1168.5 ±335.8	951.7 ±234.1 insig.
6	2.75 mM 5 mg ATP/3ml	2477.1 ±298.4	1818.7 ±256.4 sig. decrease $P < 0.01$	1103.6 ±246.1	845.2 ±177.9 insig.
8	5.5 mM 10 mg ATP/3ml	2464.8 ±268.0	1047.6 ±265.1 sig. decrease $P < 0.01$	1279.4 ±121.3	612.1 ±104.5 sig. decrease $P < 0.01$

These results are in accord with the results obtained by *Koji* et al. (20). They found that ATP in lower concentrations accelerated oxygen consumption while higher concentrations suppressed oxygen consumption of cerebral cortex in vitro. This may also throw light on our results concerning the effect of ATP on whole animal. Thus the depressant action of the two large doses of ATP is in accordance with the effect of ATP on the basal metabolic rate of the rat (35). While the potentiating effect of the small dose of ATP resemble the effect of intravenous infusion of ATP by the slow drip method in case or the dog (34).

The oxygen uptake of either muscle or liver slices and homogenate was significantly decreased at the second hour compared with the first hour both in the presence or the absence of ATP.

This may be due to the decline in the concentration of the substrate as a result of the metabolism of the respiring tissue or elements.

Chance and *Williams* (6) pointed out that the liver content of adenine and its phosphorylated derivatives and the oxygen consumption varied with the nutritional state. Thus the oxygen consumption of liver slices from fasted rats were low.

In physiologically intact preparations the rates of respiration and phosphorylation are interdependent. The rate of respiration can be limited by the rate of phosphorylation. If the latter is inhibited by the lack of suitable substrates (ADP or phosphate), respiration is also inhibited (21).

Table 4. Effect of different concentrations of ATP on the oxygen uptake of fresh liver slices (0.5 mm thick) at 37 °C.

No. of expt.	Conc. of ATP	Oxygen uptake in mm^3/g wet wt liver slices/hour 1st hour control	1st hour treated	2nd hour control	2nd hour treated
8	0.55 mM 1 mg ATP/3 ml	1815.1 ±260.7	1992.1 ±360.8 insig.	1369.1 ±310.1	1567.9 ±282.0 insig.
10	1.1 mM 2 mg ATP/3 ml	1861.8 ±207.0	1611.0 ±260.0 sig. decrease $P < 0.05$	1630.2 ±273.1	1302.2 ±219.3 sig. decrease $P < 0.01$
10	2.75 mM 5 mg ATP/3 ml	1739.2 ±315.2	1207.8 ±232.4 sig. decrease $P < 0.01$	1416.2 ±270.4	928.0 ±215.8 sig. decrease $P < 0.01$
10	5.5 mM 10 mg ATP/3 ml	1699.9 ±126.1	928.1 ±202.5 sig. decrease $P < 0.01$	1156.8 ±148.5	553.6 ±137.0 sig. decrease $P < 0.01$

Klingenberg (17) has extended the concept of respiratory control by ATP and inorganic phosphate to include the ATP/ADP ratio or phosphorylation potential as an important factor in control of respiration. This concept was later supported by the demonstration of respiratory inhibition by ATP (19). The evidence for the existence of this mechanism came from observations on mitochondria from skeletal muscle, whose respiration was inhibited by the addition of ATP (17, 18).

ATP can partially inhibit respiration that has been fully stimulated by prior addition of ADP. At the same time, the pyridine nucleotides change to a more reduced state. This reduction of the pyridine nucleotides, which occurs parallel with the inhibition of respiration, shows that ATP by reversing oxidative phosphorylation exerts an effect that opposes that of ADP. It can thus be seen that the activity of respiration depends on the ratio ATP/ADP.

ATP is inhibitor-competitive with DPN of the enzyme from heart and liver and *Ehrlich* ascitis tumor (32).

They suggested that the inhibition of ATP is due partly to chelation of activating divalent cations. Chelation of Mg^{2+} has been considered as the single explanation of the inhibition by ATP of the DPN-linked enzyme from rat heart extracts (12).

Table 5. Effect of different concentrations of ATP on the oxygen uptake of fresh liver homogenate at 37 °C.

No. of expt.	Conc. of ATP	Oxygen uptake in mm^3/g wet wt liver homogenate/hour 1st hour control	1st hour treated	2nd hour control	2nd hour treated
7	0.275 mM 0.5 mg ATP/3 ml	5343.27 ±293.51 insig.	5673.2 ±391.3 insig.	4661.2 ±242.7	4686.9 ±281.1 insig.
10	0.55 mM 1 mg ATP/3 ml	6000.8 ±498.7	2701.3 ±451.1 sig. increase $P < 0.01$	3612.4 ±703.3	4819.6 ±312.3 sig. increase $P < 0.01$
9	1.1 mM 2 mg ATP/3 ml	5310.3 ±134.6	5532.1 ±385.1 insig.	4019.0 ±286.5	3919.9 ±356.5 insig.
13	2.75 mM 5 mg ATP/3 ml	6228.3 ±684.8	5525.4 ±562.4 sig. decrease $P < 0.01$	4515.5 ±761.1	2622.3 ±660.5 sig. decrease $P < 0.01$
14	5.5 mM 10 mg ATP/3 ml	5894.0 ±470.5	1753.3 ±734.8 sig. decrease $P < 0.01$	4321.1 ±497.6	410.7 ±442.8 sig. decrease $P < 0.01$

A similar combination of chelation and competition with coenzyme accounts for the inhibition by ATP of a TPN-linked isocitrate dehydrogenase (23). Yet a different type of inhibition by ATP involving interaction with a protein site other than substrate loci (and not competition with coenzyme or chelation of metal) has been observed with TPN-linked enzyme (24).

In the present investigation the only significant potentiating effect of ATP on the oxygen uptake was noticed in fresh liver homogenate in presence of 0.55 mM ATP concentration. This dose was considered the optimal dose in increasing the uptake of oxygen by the fresh liver homogenate.

It is to be noted that this same dose was the optimal dose that stimulated the oxygen uptake by the fresh kidney slices (8).

Working on the effect of ATP on fatigue of neuromuscular transmission, *Talaat* et al. (36) found that the same dose was the optimal dose exerting an antifatigue effect as well as potentiating the recovery of neuromuscular transmission after complete neuromuscular block. Large dose of ATP above the optimal dose resulted in a reversal of the beneficial effect and promoted the fatigue process. This was explained by a decline in the rate of splitting of ATP.

Heinz and *Holton* (13) gave similar results in fibre models. They reported the dependence of the splitting of ATP on its concentration. They also reported that supraoptimal concentration of ATP results in a decrease in both the tension of the fibres models and in the rate of splitting of ATP.

In the present investigation a 10% homogenate in 0.25 M sucrose was prepared. This medium was shown (30) to be excellent for the preparation of homogenates with mitochondria, as its conditions appeared to approach that of the intracellular state.

Moreover, 0.25 M sucrose is considered the best medium for the investigation in which the maintenance of ATP is required (26).

The reaction conditions suitable for studying tissue homogenates are generally those suitable for studying cell fraction. So while in tissue slice technique the assay medium must resemble that of extracellular fluid with its sodium, chloride and calcium ions the reaction medium for tissue homogenate must represent an intracellular medium. Thus it is recommended by *Polter* (27) to avoid the above-cited ions or to include them in very small amounts.

Comparing the oxygen uptake of fresh liver or muscle tissue when prepared as homogenate with that of the slices, it was found that uptake by homogenate amounted to as much as 2–3 times that of the slices. Similarly the results of *Dorria-El-Agouri* (8) showed that O_2 uptake by *Kidney* homogenate amounted to as much as 3 times that of the slices.

Homogenization made possible the extensive fragmentation of the cell membranes with little damage for the cell contents, namely nuclei, mitochondria, microsome and soluble enzymes.

Ernster and *Navazio* (10) showed that about 80% of the TPN enzyme activity of rat liver is in the soluble portion of the homogenate whereas only 12% is associated with mitochondria. However, DPN-linked enzyme is located exclusively in mitochondria.

The variation in conditions of tissue slices and homogenate may throw light on the increased O_2 uptake of the liver and muscle homogenate compared with that of the slices.

Concerning the effect of ATP on the O_2 uptake of the liver there is discrepancy between liver slices and homogenate. Thus 1.1 mM ATP caused no significant change on the oxygen uptake by the fresh liver homogenate while the same concentration caused a significant decrease on the oxygen uptake of liver slices. Also 0.55 mM ATP caused a stimulatory effect on the oxygen uptake of liver homogenate and insignificant change in case of liver slices (table 4, 5).

This may be due to the different action of ATP with respect to the enzyme adenyl cyclase in homogenate and slices of different tissues.

In studies with whole washed cells (avion erythrocytes), *Davoren* and *Sutherland* (7) found that in contrast to broken cell systems the addition of ATP and magnesium ions did not enhance the accumulation of cyclic AMP.

However, homogenate prepared from a number of tissues, including rat liver, and pigeon erythrocytes are able to synthesize cyclic AMP when they are incubated with adenosine triphosphate and Mg^{++} (33).

The altered level of intracellular cyclic AMP can also change the metabolic behaviour of the cell (29).

Cyclic AMP and related stimulants could accelerate rate-limiting reaction in the glycolytic pathway or in the tricarboxylic acid cycle, the phosphorylation of fructose-6-phosphate is a possible site (22). Phosphofructokinase is the enzyme which catalyses the reaction.

For many years ATP has been considered in terms of its role as an intracellular energy source. However, there is growing recognition that ATP and related purine nucleotides have potent extra-cellular actions on excitable membranes and that these may be involved in physiological regulatory mechanisms. The high sensitivity of smooth and cardiac muscle cell membranes to purine nucleotides was first reported in 1929 (9).

Holton (15) suggested that ATP was released during antidromic stimulation of sensory nerves, and it has been proposed that adenosine or (ATP) is a physiological regulator of blood flow in coronary, renal, skeletal muscle and cerebral vascular beds (4).

Recently *Burnstock* (2) put forward the purinergic nerve hypothesis, when evidence was presented that a purine nucleotide, probably ATP, was the principal transmitter released from the non-adrenergic, non-cholinergic "purinergic" nerves supplying the gastrointestinal tract (5).

Since that time a considerable body of evidence has been accumulated in support of the hypothesis, and the presence of purinergic nerves has been proposed in a variety of other organs (3).

The finding that exogenously applied ATP mimics the action of the nerve-released transmitter provides further support for the purinergic nerve hypothesis (4).

It is concluded therefore that the wide-spread actions of ATP at the cellular level, its effect on the mitochondria and on plasma membranes, its interactions with various enzymes systems make the effect of ATP on the oxygen consumption unpredictable.

However, the discovery of purinergic nerve hypothesis will help in the understanding of many of the actions of ATP.

Summary

The effect of exogenous adenosine triphosphate on the oxygen uptake of rat diaphragm and liver slices and homogenate were studied in normal rats using Warburg Manometric Technique. Five concentrations of ATP were used, namely 0.275, 0.55, 1.1, 2.75, and 5.5 mM ATP.

ATP in small concentrations showed no significant changes in oxygen uptake of rat diaphragm and muscle homogenate or liver slices, but the oxygen uptake of liver homogenate was significantly increased in presence of 0.55 mM ATP while large concentrations caused depression in oxygen uptake of rat diaphragm and liver tissue whether it was sliced or homogenate.

The depressant action of large concentrations of ATP on oxygen uptake by liver and muscle tissues is explained by the finding that the activity of respiration is dependent on the ratio of ATP/ADP.

The stimulatory effect of the small concentration of ATP in case of liver homogenate and the absence of its effect in case of liver slices may be due to the different action of ATP with respect to adenyl cyclase in homogenate and slices.

The oxygen uptake of homogenate was significantly greater than that of slices. This is presumably due to the greater excess of the intracellular respiratory mechanisms in the homogenate than in the slices.

However, the recent discovery of the purinergic nerve hypothesis and the finding that exogenously applied ATP mimics the action of the nerve-released transmitter will greatly improve the understanding of the action of ATP.

References

1. *Atkinson, D. E.*, Science **150,** 851 (1965). – 2. *Burnstock, G.*, Pharmacol. Rev. **24,** 509 (1972). – 3. *Burnstock, G.*, Purinergic transmission. In: Handbook of Psychopharmacology, Vol. 5 (*Inverson, L. L.* and *Synden, S. H.*, eds.). pp. 131–194 (New York 1975). – 4. *Burnstock, G.*, The purinergic nerve hypothesis. In: Purine and Pyrimidine Metabolism, Ciba Foundation Symposium No. 48. pp. 295–314 (Amsterdam 1977). – 5. *Burstock, G., T. Cocks, L. Kasakov, H. Wong*, Europ. J. Pharmacol. **49,** 145 (1978). – 6. *Chance, B., G. B. Williams*, J. Biol. Chem. **217,** 383 (1955). – 7. *Davoren, P. R., E. W. Sutherland*, J. Biol. Chem. **238,** 3009 (1963). – 8. *Dorria El-Aggowri*, Thesis for M. D. Physiol. Studies on Effect of cold storage on metabolism of the kidney. Physiol. Dept. Facul. of Med. Cairo Univ. (1970). – 9. *Drury, A. N., A. Szent-Györgyi*, J. Physiol. (Lond.) **68,** 213 (1929). – 10. *Ernster, L. F., Navazio*, Exp. Cell. Res. **11,** 483 (1956). – 11. *Fuhrmann, F. A., G. T. Fuhrmann, A. F. Douglas, H. F. Jane*, Amer. J. Physiol. **201,** 231 (1961). – 12. *Goebell, H., M. Klingenberg*, Biochem. Z. **340,** 441 (1964). – 13. *Heinz, E., F. Holton*, Z. Naturforschg. **76,** 386 (1952). – 14. *Himms-Hagen, J.* Pharmacol. Rev. **19,** 368 (1967). – 15. *Holton, P. J.*, Physiol. (Lond.) **45,** 494 (1959). – 16. *Kayne, H. L., N. Taylor, N. R. Albert*, Metabolism **12,** 1012 (1963). – 17. *Klingenberg, M., P. Schollmeyer*, Biochemische Zeitschrift **333,** 335 (1960). – 18. *Klingenberg, M.*, Symposium on biol. structure and function, Academic Press **2,** 227 (New York 1961). – 19. *Klingenberg, D. M.*, Angew. Chemie **3,** 54 (1964). – 20. *Koji, M., T. Harada, R. Nishimura, M. Kurata, K. Nishibara*, J. Kurume Med. Assoc. **3,** 664 (1971). – 21. *Lardy, H. A.*, Proceedings of the Third International Congress of Biochemistry, Brussels (1955) p. 28 (New York 1956). – 22. *Mansour, T. E.*, Pharmacol. Rev. **18,** 173 (1966). – 23. *Marr, J. J., M. M. Weber*, J. Biol. Chem. **243,** 4973 (1968). – 24. *Marr, J. J., M. M. Weber*, J. Biol. Chem. **244,** 2503 (1969). – 25. *Martin, A. W., F. A. Fuhrman*, (1955). Quoted from Amer. J. Physiol. **201,** 231 (1961). – 26. *Polter, U. R., P. Siekevitz, H. C. Simonson*, J. Biol. Chem. **205,** 893 (1953). – 27. *Polter, U. A.* (1959). Quoted from Monometri Technique, 4th Edition (1964) by

Umbreit, W. W., R. H. Burris, and *J. F. Stauffer.* – 28. *Robinson, J. R.,* Biochem. J. **45,** 68 (1949). – 29. *Robinson, G. A., R. W. Butcher, E. W. Sutherland,* Cyclic AMP. Academic Press (New York 1971). – 30. *Schneider, W. C., G. H. Hogeboom,* J. Biol. Chem. **183,** 123 (1950). – 31. *Stadie, W. C., B. C. Riggs,* J. Biol. Chem. **154,** 687 (1944). – 32. *Stein, A. M., S. K. Kirkman, J. H. Stein,* Biochemistry **6,** 3197 (1967). – 33. *Sutherland, E. W., T. W. Ball, T. Menon,* J. Biol. Chem. **237,** 1220 (1962). – 34. *Tahani H. Mikhail, Kairia M. Ibrahim, Y. A. Habib, M. Talaat,* Ain Shams Med. J. Under publication. – 35. *Tahani H. Mikhail, Samia S. Rizk, Y. A. Habib, M. Talaat,* Bull. NRC., Egypt (in press). – 36. *Talaat, M., Y. A. Habib, A. W. El-Shihy, Y. Abdel-Rahman, I. El-Bagouri,* UARJ Physiol. Sci. **1,** 39 (1965).

Authors' address:

Tahani H. Mikhail, National Research Centre, Dokki, Cairo (Egypt)