EFFECT OF EXTRACELLULAR ATP AND Ca^{2+} AND Mg^{2+} IONS ON OSMOTIC PROPERTIES OF CEREBRAL CORTICAL SYNAPTOSOMES

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UDC 612.823.5.014.462.1-06:
616.398.145.1

KEY WORDS: mediator; synaptosome; ATP; swelling.

Evidence has recently been obtained that ATP is a neurotransmitter, at least in the specialized purinergic synapses of smooth muscle of the vertebrate gastrointestinal tract and also in smooth muscles of the bladder and lung [8], where it exerts an inhibitory action and induces hyperpolarization. It has been suggested [14] that ATP has a mediator action in the brain also. On the other hand, such well-studied mediators as acetylcholine and nonadrenalin have been shown to occur in synaptic vesicles together with considerable amounts of ATP [5]. ATP is secreted into the synaptic space during electrical stimulation of nerve-ending membranes [11]. A role of ATP in the combined superprecipitation of proteins in vesicles and presynaptic membranes has been demonstrated [4]. At this stage ATP participates in the mechanism of mediator secretion and behaves as an intracellular factor. However, secretion of ATP itself from the nerve ending, mentioned above, must also be taken into account. This extracellular ATP may have a modulating influence on the state of synapses. It is therefore necessary to study the effect of extracellular ATP on different properties of whole synaptosomes from the cerebral cortex.

EXPERIMENTAL METHOD

Individual fractions of synaptosomes from the rat cerebral cortex were obtained by De Robertis' method [10] in Globov's modification [6]. Purified heavy and light synaptosomes and mitochondria were suspended in medium containing 0.32 M sucrose and 10 mM Tris-HCl, pH 7.2.

The state of the synaptosomes was judged by their osmotic properties. These were determined spectrophotometrically by measuring the rate of swelling of the synaptosomes and mitochondria in different media [9]: for this purpose 2.8 ml of the corresponding medium and 0.2 ml of a suspension of synaptosomes or mitochondria were poured into a standard cuvette, the mixture was stirred and, starting at 20°C, absorption was quickly recorded at 520 nm (E_{520}). After 6 min 0.1 ml of a solution of ATP or AMP to the final concentration indicated in the captions to the figures was added. In control experiments 0.1 ml of Tris-HCl, pH 7.2, was added at the same time (the solution in which the ATP-Na_2 solution was made up).

The resulting swelling was recorded in the decrease in optical density of the supension in the course of 10 min. Graphs of E_{520} as a function of logarithms of time were plotted. For convenience of comparison of the swelling curves, values of optical density in each experiment were converted to relative values [E_{520}], so that the last value of the optical density before addition of the test factors was unity.

EXPERIMENTAL RESULTS

It was shown previously that on linearization of the swelling curves in isoosmotic KCl solution by drawing the graph of E_{520} as a function of log t, at a certain moment a sharp change takes place in the rate of swelling of the light and heavy synaptosomes and mitochondria [2]. As a result, the process can be clearly divided into two stages, the second of which is characterized by a much faster rate of swelling. To study the effect of ATP, AMP, and Ca^{2+} and Mg^{2+} cations on the osmotic properties of synaptosomes and mitochondria, the substances mentioned above were added in the second, more active, stage of swelling. Comparison of the curves after conversion of E_{520} into relative values [E_{520}], where E_{520} is taken as unity at the moment of addition of the test factors, is particularly demonstrative.

Department of Biochemistry, M. V. Lomonosov Moscow University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 2, pp. 150-153, February, 1981. Original article submitted July 8, 1980.

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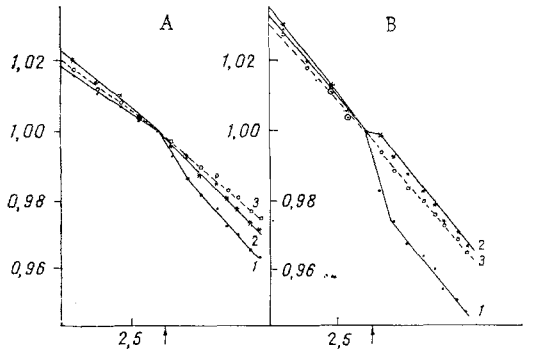

Fig. 1. Effect of ATP and AMP in a concentration of 2.6 mM on rate of swelling of light synaptosomes (A) and mitochondria (B) from rat cerebral cortex in 0.15 M KCl and 0.02 M Tris-HCl, pH 7.2. Abscissa, log t; ordinate [E_{520}]. 1) ATP, 2) AMP, 3) control. Time of addition of ATP and AMP indicated by arrow.

The effect of ATP was studied in concentrations close to its mean brain levels, namely 2 mM [3], or lower. It will be clear from Fig. 1, A and B that ATP, unlike AMP, in the presence of 0.15 M KCl and 0.02 M Tris-HCl, pH 7.2, caused an initial rise in the rate of swelling of light synaptosomes and mitochondria, but later the mitochondria continued to swell at the former rate whereas the light synaptosomes swelled at a rather faster rate. With ATP and AMP concentrations 100 times less, no difference was found between swelling of the synaptosomes and mitochondria.

Addition of ATP in the presence of Mg^{2+} led to rapid shrinking of the light synaptosomes (Fig. 2A) and mitochondria (Fig. 2B), after which their behavior differed: The synaptosomes swelled again at the former rate whereas the mitochondria swelled much more slowly.

Mg^{2+} and Ca^{2+} also caused rapid shrinking of the particles of all fractions studied, followed by swelling at the same rate.

The study of the osmotic properties of the synaptosomes in Krebs–Ringer solution [7], which to some extent simulates the salt and carbohydrate composition of the intercellular fluid, is particularly interesting. Addition of ATP, unlike AMP, to light synaptosomes (Fig. 3A) and mitochondria (Fig. 3B) in Krebs–Ringer solution led to an increase in the rate of swelling of both light synaptosomes and mitochondria; the degree of swelling was more clearly dependent on ATP concentration in the case of light synaptosomes than of mitochondria.

With respect to their behavior in isoosmotic KCl solution and in Krebs–Ringer solution heavy synaptosomes occupy an intermediate position between light synaptosomes and mitochondria, probably due to some contamination of the heavy synaptosomal fraction by mitochondria [6].

When the results are evaluated the significant difference between the scheme of the present experiments, in which ATP acted from outside on the intact synaptosome, and previous experiments in which the action of ATP within the nerve ending on contractile proteins of the vesicles, mixed with preparations of presynaptic membranes [5, 12], was simulated, must be emphasized. In other words, the present experiments simulated a situation in which ATP was secreted either from purinergic nerve endings of from adrenergic and cholinergic nerve endings as a satellite of the mediators [13, 15] and it acted thereafter as an extracellular factor, with a modulating action on the state of the other nerve endings and synapses. Under these circumstances modulation could take place either by the classical mechanism — on account of interaction with a certain specific ATP receptor or on account of its direct action on the contractile proteins of the membrane.

On the basis of these results it is impossible to do more than simply record the fact of a change in the state of the membrane of intact synaptosomes under the influence of ATP in concentrations close to those actually attainable in brain tissues.

The changes discovered in the rate of swelling of synaptosomes are only one of the possible criteria of changes in the state of the nerve ending under the influence of extra-

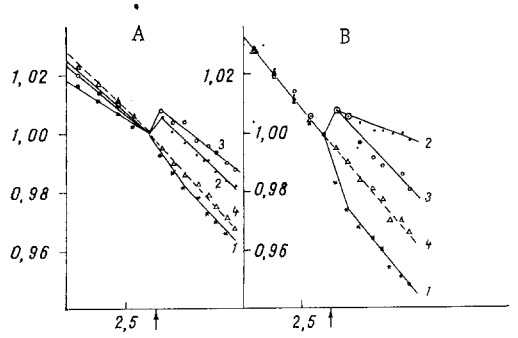

Fig. 2

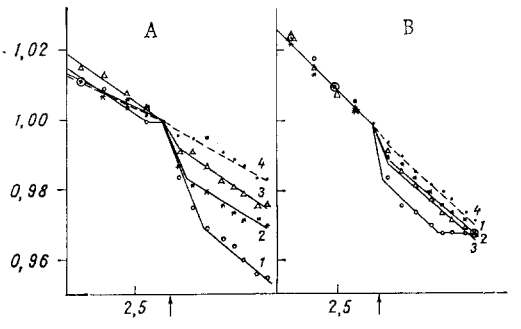

Fig. 3

Fig. 2. Effect of ATP, ATP–Mg^{2+} complex, and Mg^{2+} on rate of swelling of light synaptosomes (A) and mitochondria (B) from rat cerebral cortex in 0.15 M KCl and 0.02 M Tris-HCl, pH 7.2. 1) ATP in concentration of 2.6 mM; 2) ATP (2.6 mM) with Mg^{2+} (5.2 mM); 3) Mg^{2+} (5.2 mM); 4) control. Remainder of legend as to Fig. 1.

Fig. 3. Rate of swelling of light synaptosomes (A) and mitochondria (B) of rat cerebral cortex in Krebs–Ringer solution, pH 7.4, as a function of ATP concentration. 1) 5.2 mM ATP, 2) 0.35 mM, 3) 0.05 mM, 4) control. Remainder of legend as to Fig. 1.

cellular ATP. It is important to note that under certain conditions a significant difference was found between this process and reactions of the mitochondria to ATP: ATP in the presence of Mg^{2+} and KCl slowed swelling after the first rapid response of shrinking only in the case of mitochondria. Reactions of synaptosomes to extracellular ATP thus present unique features.

The data described above agree with the view that ATP plays a polyfunctional role in the CNS: Besides participating in reactions requiring expenditure of energy, it can also play the role of mediator or modulator.

The noncyclic purine nucleotides are possibly among the phylogenetically oldest of the mediators and modulators, and it is this which gives particular interest to the study of the mechanisms of their action [1].

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