EFFECT OF ADENOSINE-5'-O-(β,γ-DICHLOROMETHANE) TRIPHOSPHATE ON ATP RECEPTORS IN RAT SENSORY NEURONS

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ATP receptors, the activation of which causes the appearance of inward ionic currents, have been described in rat sensory neurons [1]. It has been shown that β,γ-bromomethane-ATP, unlike β,γ-methylene-ATP, is a competitive blocker of these receptors [2]. It was accordingly decided to study the pharmacologic activity of disubstituted halogen derivatives of β,γ-methylene-ATP.

EXPERIMENTAL METHOD

Experiments were carried out on 1-3-day fixed cultures of rat ganglion nodosum neurons [3]. The experimental conditions included intracellular perfusion and membrane voltage clamping, with the use of glass micropipets [2]. The extracellular solution contained (in mM): NaCl 140, KCl 1, $MgCl_2$ 1, $CaCl_2$ 3, HEPES-NaOH buffer 10 (pH 7.4). The intracellular solution contained (in mM): KF 110, Tris-HF buffer 20 (pH 7.3). The test substances were applied to the neuron by the rapid concentration jump method [2]. The holding potential was −90 mV. Adenosine-5'-O-(β,γ -dihalogenmethane) triphosphates (β,γ -$CHal_2$-ATP) were synthesized by Dr. G. M. Blackburn of Sheffield University (Great Britain) and N. B. Tarusova from the Institute of Molecular Biology, Academy of Sciences of the USSR.

EXPERIMENTAL RESULTS

β,γ-CBr_2-ATP and β,γ-CCl_2-ATP, in concentrations of 4×10^{-4} M, did not induce inward currents in neurons of the rat ganglion nodosum sensitive to ATP. Meanwhile the amplitude of ATP-activated currents was reversibly reduced in the presence of these substances. This effect was weakened with an increase in ATP concentration. Analysis of the data between Lineweaver–Burk coordinates (Fig. 1) shows that these ATP analogs are competitive blockers of ATP receptors. The effectiveness of blocking increased in the following order: β,γ-CBr_2-ATP<β,γ-CHBr-ATP<β,γ-CCl_2-ATP. β,γ-CCl_2-ATP is the most effective competitive blocker of this receptor yet known. The inhibition constant for this substance, calculated from the change in the apparent dissociation constant for ATP, is about 2×10^{-5} M.

β,γ-CF_2-ATP, in a saturating concentration (2×10^{-4} M), activates inward ionic currents with very slow desensitization kinetics,and with an amplitude almost an order of magnitude less than the amplitude of the current activated by ATP in the same concentration (Fig. 1), in neurons sensitive to ATP. The dissociation constant for this substance is about 2.5×10^{-5} M. If ATP receptors are desensitized by prolonged application of ATP (10^{-5} M), the response to β,γ-CF_2-ATP disappears. On combined application with ATP, β,γ-CF_2-ATP competitively reduces ATP-activated currents with an inhibition constant of approximately 2×10^{-5}–3×10^{-5} M. It follows from the facts described above that β,γ-CF_2-ATP and ATP interact with the same receptor, but β, γ-CF_2-ATP is partial agonist of ATP receptors.

β,γ-CH_2-ATP is an agonist of ATP receptors. Replacement of a hydrogen atom of the β,γ-methylene group of β,γ-CH_2-ATP by various halogens causes the agonist to be converted (depending on the radius of the halogen atom) either into a partial agonist or into a competitive

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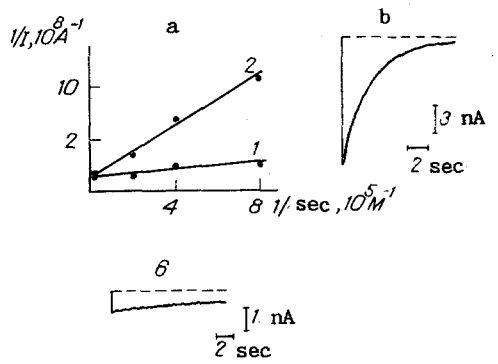

Fig. 1. ATP-activated currents in sensory neurons. a) Dose–effect dependence for ATP (1) and effect of 10^{-4} M β,γ-CCl_2-ATP on it (2); b) current activated by ATP (10^{-5} M); c) current activated by β,γ-CF_2-ATP (2×10^{-4} M).

blocker. This confirms once again the previous hypothesis that the β- and γ-phosphate groups of the ATP molecule play an important role in activation of ATP receptors [2].

In the study of ATP-ergic transmission two ATP analogs are currently used: α,β-methylene-ATP and arylazidoaminopropionyl-ATP [5]. These compounds are agonists of ATP receptors. The first of them is relatively resistant to enzymic hydrolysis, and if used for a long time, desensitizes ATP receptors. The second binds covalently to the receptor protein on irradiation by visible light. ATP receptors are known to emerge very slowly from a state of desensitization [4] and for that reason the use of α,β-CH_2-ATP is not always convenient. Arylazidoaminopropionyl-ATP acts irreversibly and, evidently, nonspecifically. Accordingly, to study the physiological role of ATP receptors, it is more acceptable to use competitive blockers and, in particular, β,γ-CCl_2-ATP, which is sufficiently effective and relatively resistant to enzymic hydrolysis.

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