EFFECT OF GUANINE NUCLEOTIDES AND HYPERBARIC OXYGENATION ON CARDIAC ADENYLATE CYCLASE ACTIVITY IN RABBITS WITH MYOCARDIAL HYPERTROPHY

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UDC 616.127-007.64-092.9-07:616.12-008.931:577.152.633-02:615.835.12

KEY WORDS: adenylate cyclase; rabbit heart; myocardial hypertrophy; guanine nucleotides; hyperbaric oxygenation.

The hormonal regulation of myocardial function and metabolism is mediated through cyclic nucleotides. As a rule an increase in the cAMP concentration is accompanied by the development of positive inotropic and chronotropic effects on the myocardium. When hormonal regulation of the heart muscle is disturbed changes arise both in the cAMP concentration and in activity of enzymes of cAMP synthesis and breakdown: adenylate cyclase and phosphodiesterase, whereas if hormones are present in excess, the sensitivity of the adenylate cyclase system to hormonal action is reduced ("desensitization"). These disturbances may result from or lie at the basis of several cardiovascular diseases [2, 3, 10, 11, 13].

There are data in the literature on desensitization of the adenylate cyclase system not only of heart tissue, but also of tissues of other organs, to hormonal action [9, 12, 14]. It has been suggested that important participants in the development of desensitization and resensitization of the adenylate cyclase system are guanine nucleotides, which are involved in signal transmission from hormone receptors to enzyme.

The writers showed previously that changes in the cAMP concentration, basal adenylate cyclase activity, and desensitization of adenylate cyclase to adrenalin take place in allergic myocarditis [1] and compensatory hypertrophy of the myocardium [5]. Partial normalization of the disturbed balance of the system for cAMP metabolism has been obtained in myocardial hypertrophy by means of hyperbaric oxygenation (HBO), a mode of treatment which can cause modifications to the neurohormonal regulation of myocardial function and metabolism [4-6].

The object of this investigation was to study the role of guanine nucleotides and of HBO in desensitization and resensitization of adenylate cyclase in the heart of rabbits with myocardial hypertrophy.

EXPERIMENTAL METHOD

The model of compensatory myocardial hypertrophy in rabbits used in the experiments and the method of HBO were described previously [5]. Methods of isolation of partially purified membrane preparations and of determining adenylate cyclase activity also were described in [1]. The ATP concentration in the incubation medium was 0.1 mM and concentrations of GTP and guanylyl-5'-imidodiphosphate (GIDP) were 10^{-5} M. The results of five to six similar experiments are given in Figs. 1 and 2.

EXPERIMENTAL RESULTS

The study of the effect of guanine nucleotides on adenylate cyclase activity in the presence and absence of adrenalin and under normal and pathological conditions showed (Fig. 1) that GTP virtually does not activate adenylate cyclase without adrenalin, whereas its unhydrolyzed analog GIDP activates the enzyme many times over. In the presence of adrenalin guanine nucleotides activate adenylate cyclase much more strongly, especially GIDP,

Laboratory of Enzymology, Academy of Medical Sciences of the USSR. Department of Hyperbaric Oxygenation, All-Union Scientific Center of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 2, pp. 17-19, February, 1983. Original article submitted June 1, 1982.

0007-4888/83/9502-0179$07.50

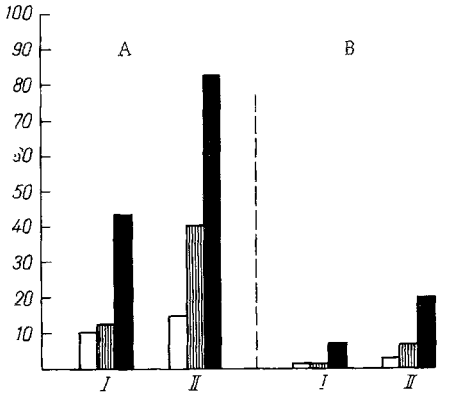

Fig. 1. Effect of guanine nucleotides on myocardial adenylate cyclase activity. Ordinate, adenylate cyclase activity (in moles cAMP/mg protein/min). A) Normal state, B) hypertrophy. I) Without adrenalin, II) adrenalin added. Unshaded columns — basal activity, vertically shaded — in presence of GTP, black columns — in presence of GIDP.

whereas without them the hormone alone has virtually no effect on the activity of this enzyme.

In myocardial hypertrophy levels of both basal adenylate cyclase activity and of activity activated by guanine nucleotides were considerably below normal. Meanwhile desensitization of adenylate cyclase to adrenalin was observed. Absence of activation of the enzyme by adrenalin under pathological conditions within the concentration range from 10^{-7} to 10^{-4} M confirms desensitization of adenylate cyclase to the hormone.

Data on the effect of HBO and guanine nucleotides on adenylate cyclase activity in myocardial hypertrophy are shown in Fig. 2. Under the influence of HBO both basal activity of the enzyme and its activation by guanine nucleotides were increased and the sensitivity of the enzyme to adrenalin also was restored. Under these conditions, in the presence of guanine nucleotides adrenalin, by activating adenylate cyclase, brought the activity of the enzyme up to the normal level.

It is nowadays considered that the adenylate cyclase system consists of a hormone receptor, located on the outer side of the membrane, of regulatory GTP-dependent proteins which possess hormone-dependent GTPase activity (so-called G proteins), and the catalytic elements of adenylate cyclase proper [8, 15, 16]. To convert adenylate cyclase into a state with high catalytic activity, the simultaneous presence of the hormone and of guanine nucleotides is necessary in order to permit interaction of the enzyme with the hormonal receptors and GTP-binding protein. The role of the hormone—receptor complex in adenylate cyclase activation consists of facilitating substitution of GDP, bound with G-protein, for GTP. It has been shown that on binding GTP or its analog, G-protein forms a complex with the catalytic subunit of adenylate cyclase and thereby converts the enzyme into the active state. Since the G-protein possesses GTPase activity, it is suggested that hydrolysis of GTP to GDP leads to disintegration of the catalytically active complex [15].

Desensitization of the adenylate cyclase system to hormonal action is due to desensitization of the membrane receptors in the presence of an excess of hormone and to the change in functional state of proteins forming the adenylate cyclase complex that takes place under these circumstances. An important role in this process is played by the G-protein and, correspondingly, by guanine nucleotides [16].

The results of this investigation confirm the role of G-protein in activation of cardiac adenylate cyclase under both normal and pathological conditions, for guanine nucleotides increase activity of the enzyme (Figs. 1 and 2). In all cases activation by GTP was lower than by GIDP, due to hydrolysis of the GTP in the regulatory center of adenylate cyclase [7, 8, 15, 16]. Under normal conditions in the presence of adrenalin activation of the enzyme by

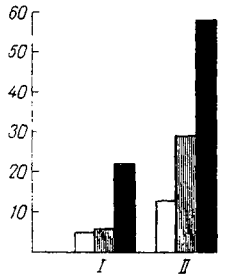

Fig. 2. Effect of guanine nucleotides on adenylate cyclase activity of hypertrophied myocardium under the influence of HBO. Legend as to Fig. 1.

guanine nucleotides reaches its highest level on account of conversion of adenylate cyclase into a state with high catalytic activity.

During hypertrophy of the heart muscle sensitivity of the enzyme to adrenalin was considerably reduced. Meanwhile a considerable decrease in the activating effect of GTP and GIDP also was found (Fig. 1), evidence of a change in the functional state of the regulatory G-protein. This change may be due both to an increase in hydrolase activity of the G-protein and to disturbance of its ability to bind guanine nucleotides. As a result the activating effect of GTP and GIDP on adenylate cyclase is reduced.

Normalization of adenylate cyclase activity by HBO under pathological conditions indicates resensitization of the adenylate cyclase system to hormonal action.

Disturbances of functions of the G-protein in pathology are thus abolished by HBO. This may be the result of restoration of the ability of G-protein to bind guanine nucleotides, as well as possible oxidation of its sulfhydryl groups, leading to a decrease in GTPase activity and, consequently, a return toward normal adenylate cyclase activity.

The results are evidence that in myocardial hypertrophy guanine nucleotides participate both in the desensitization of adenylate cyclase to hormonal action and in its resensitization during HBO therapy.

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