DEPENDENCE OF INTRINSIC ACTIVITY OF THE β-ADRENERGIC AGONIST ISOPROTERENOL ON PLASMA MEMBRANE PERCOLATION PROPERTIES

A. R. Kazarov, A. A. Rozenkrants, and A. S. Sobolev

UDC 615.217.22.015.21].015.44.076.9

KEY WORDS: isoproterenol, β-adrenoreceptor, regulatory N protein, adenylate cyclase, "liquid" lipids.

To activate adenylate cyclase, binding of the agonist with the corresponding receptor and subsequent formation of a hormone—receptor—N_S-protein complex are essential. If GTP is present in the medium this complex quickly breaks down and the α-subunit of the N_S-protein forms a complex with adenylate cyclase and activates it [1, 14]. In the absence of guanyl nucleotides, however, the complex can be found on the basis of increased affinity for the agonist. During activation displacement of proteins of the adenylate cyclase system takes place in the membrane [2, 4, 13]. Effective transposition of proteins in the plane of the membrane is effected in "liquid" (in the liquid-crystalline state) lipids. A change in the fraction of "liquid" lipids will therefore lead to a change in the membrane fraction accessible for transposition, i.e., it may cause a change in the macrostructure of the membrane. It is thus important to determine whether changes in membrane macrostructure can modulate the response of the adenylate cyclase system to the action of β-adrenergic agonists and, if they can, within what limits. To classify drugs by effectiveness the concept of "intrinsic activity" [3, 8], reflecting the effectiveness of similar hormone-like substances relative to that which exhibits maximal action, is used. Intrinsic activity of several β-adrenergic agonists, assessed on the basis of the effectiveness of their activation of adenylate cyclase, is known to correlate with the ability of these agonists to induce the formation of a triple complex: agonist—receptor—N_S-protein [9].

The aim of this investigation was to study correlation between changes in the intrinsic activity of the β-adrenergic agonist isoproterenol and the ability of β-adrenoreceptors to form a triple high-affinity complex depending on a change in the fraction of "liquid" lipids in the plasma membranes of rat reticulocytes.

EXPERIMENTAL METHOD

Induction of reticulocytosis by phenylhydrazine was carried out as described previously [10, 13]. Incubation with phospholipase A_2, treatment with bovine serum albumin (BSA), ans subsequent centrifugations followed the method in [2, 13]. The fraction of "liquid" lipids in the membrane was determined by McConnel's method in the modification [15], using the spin probe 2,2,6,6-tetramethylpiperidyl-1-oxyl (TEMPO). Binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) with the plasma membranes was determined as described previously [13]; the ratio of the fractions of receptors in the composition of the high-affinity complex was determined from concentration dependences of displacement of ^{3}H-DHA ($2.0 \cdot 10^{-9}$ M) L-isoproterenol ("Sigma," USA). The displacement data were analyzed on the BESM-6 computer in accordance with Feldman's mathematical model [6], allowing for nonspecific binding [7]. Adenylate cyclase activity was determined by the method in [5], as described previously [13]. Protein was determined by Lowry's method [12].

EXPERIMENTAL RESULTS

Hydrolysis of membrane phospholipids by phospholipase A_2 followed by removal of the hydrolysis products from the membranes by incubation with BSA, purified from fatty acids, led to extraction of some of the phospholipids. This modification of the membranes leads to a marked decrease in the fraction of "liquid" lipids [2, 13]. It has been shown that

Laboratory of Membrane Biochemistry, Institute of Applied Molecular Biology, Ministry of Health 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. 106, No. 9, pp. 319-321, September, 1988. Original article submitted January 22, 1988.

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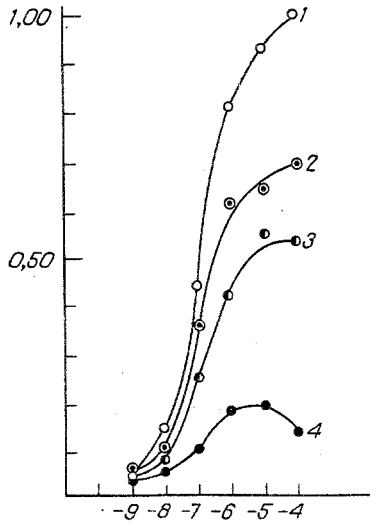

Fig. 1. Dependence of adenylate cyclase activity of reticulocyte membranes on L-isoproterenol concentration. Abscissa, log of L-isoproterenol concentration; ordinate, adenylate cyclase activity (in fractions of maximal). 1) Control membranes; 2, 3, 4) membranes with fraction of "liquid" lipids reduced by 30, 41, and 54% respectively.

with a decrease in the fraction of "liquid" lipids in the membrane the ability of β-adrenoreceptors to form complexes with high affinity for L-isoproterenol is reduced. For instance, whereas in the control the fraction of high-affinity complexes was 65%, with a reduction in the content of "liquid" lipids by 41 and 54%, their fraction fell to 34 and 10% respectively. This is evidence of depression of the ability of the receptors to form the triple complex: agonist—receptor—N_s-protein.

Reduction of the "liquid" lipid fraction is accompanied by depression of hormonal activation of adenylate cyclase (Fig. 1). Intrinsic activity of L-isoproterenol, estimated as its ability to activate adenylate cyclase in the control membranes, was taken as 1. Maximal activation of adenylate cyclase by L-isoproterenol in membranes with a reduced fraction of "liquid" lipids is therefore represented in Fig. 1 in units of intrinsic activity relative to control membranes. Reduction of the fraction of "liquid" lipids is thus accompanied by a fall in the intrinsic activity of L-isoproterenol.

The effect of treatment with phospholipase and BSA on functional integrity of the components of the adenylate cyclase system was investigated. To do this, we studied in particular binding of the labeled β-adrenergic antagonist ^{3}H-DHA with receptors, and followed it up with Scatchard plot analysis. It was found that neither the number of binding sites, reflecting the number of β-adrenergic receptors in the membranes, nor the dissociation constant of the antagonist—receptor complex changed within the limits of changes in the fractions of "liquid" lipids tested. Adenylate cyclase activity, measured in the presence of Mn^{++} ions, corresponds to activity strictly of a catalytic unit [11]. In the present investigations adenylate cyclase activity, measured in the presence of 20 mM $MnCl_2$, was unchanged by a decrease in the fraction of "liquid" lipids. Consequently, the catalytic proteins of adenylate cyclase also remained functionally intact.

Integrity of proteins of the adenylate cyclase system and their potential ability to form functional complexes after a reduction in the "liquid" lipid fraction in the membranes were tested in experiments with preliminary incubation with L-isoproterenol and the non-hydrolyzable GTP analog – guanylylimidodiphosphate (GIDP). Incubation with L-isoproterenol in the absence of guanyl nucleotides leads to the formation of long-living triple complexes in the membrane [1, 14]. After such preincubation, reduction of the "liquid" lipid fraction was found not to depress the formation of functional triple complexes. If, however, the membranes were incubated with isoproterenol and GIDP, stable complexes of N_s-protein with the catalytic unit were formed [1, 14]. Adenylate cyclase activity in these preincubated membranes, measured in the presence of GIDP, did not fall even at maximal degrees of reduction of "liquid" lipids. Consequently, a decrease in the fraction of "liquid" lipids leads

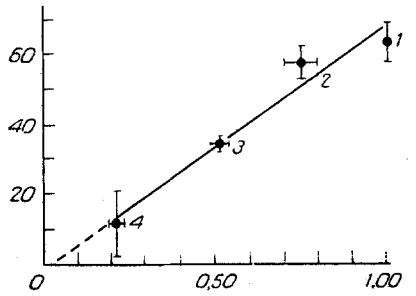

Fig. 2. Correlation between changes in characteristic activity of L-isoproterenol and number of β-receptors in composition of complexes with high affinity for L-isoproterenol on a decrease in fraction of "liquid" lipids. Abscissa, intrinsic activity of adenylate cyclase (in fractions of maximal); ordinate, fraction of β-receptors in composition of high-affinity complexes (%). Remainder of legend as to Fig. 1.

to reduction of the membrane fraction accessible for protein transposition, while proteins of the adenylate cyclase system remain functionally intact. Meanwhile the formation of complexes from protein molecules is depressed, although the latter fully preserve their potential capacity for functional interaction. Reduction of the "liquid" lipid fraction causes a change in the properties of the membrane that are essential for interaction between proteins undergoing transposition in it. As a result of the reduction of the membrane surface fraction accessible for transposition of proteins below a threshold level (the percolation threshold), the single continuous space (percolation cluster) breaks up into separate, unconnected regions [2, 13]. This, in turn, leads to a disturbance of protein interaction.

Data illustrating correlation between reduction of the fraction of high-affinity complexes and intrinsic isoproterenol activity associated with different degrees of reduction of the "liquid" lipid fraction, are illustrated in Fig. 2: the dependence is linear and can be extrapolated to the origin of coordinates.

With a decrease in the fraction of "liquid" lipids in the membranes, intrinsic activity of isoproterenol may thus decline virtually to nought. As Fig. 2 shows, these changes correlate directly with the ability of the agonist to induce agonist—receptor—N_s-protein complexes in the membrane. Consequently, changes in the macrostructure of the cell membrane may considerably modify the effectiveness of hormonal action.

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