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ISOPROTERENOL-INDUCED ADP-RIBOSYLATION OF A SINGLE PLASMA MEMBRANE PROTEIN OF CULTURED DIFFERENTIATED RL-PR-C HEPATOCYTES

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SUMMARY: We present here what we believe to be the first report of the stimulation of NAD^+-dependent ADP-ribosyltransferase activity by a hormone. Isoproterenol stimulated the ADP-ribosylation of RL-PR-C hepatocyte membranes in a concentration-dependent fashion; the effect was abolished by the β-adrenergic antagonist, propranolol. Although hepatocyte plasma membrane ADP-ribosyltransferase and adenylate cyclase activities differed in their sensitivity to isoproterenol, the kinetics of both effects were quite similar. PAGE separation of membrane proteins after ADP-ribosylation from [2,8-^{3}H-adenine]NAD^+ identified the acceptor for isoproterenol-enhanced ADP-ribosylation as the same 55,000 dalton guanyl nucleotide regulatory protein serving for both endogenous and cholera toxin-stimulated processes in these cells.

NAD^+-dependent ADP-ribosylation of guanyl nucleotide regulatory proteins is integral to the mechanism by which cholera toxin activates adenylate cyclase in avian erythrocytes and mammalian cells (1-5). Recently, Moss and coworkers described (6), and purified to homogeneity (7), a cytosolic ADP-ribosyltransferase from turkey erythrocytes that catalyzes the NAD^+-dependent ADP-ribosylation of many erythrocyte proteins and the activation of rat brain adenylate cyclase. Their work provided evidence that animal cells might employ mechanisms similar to those used by cholera toxin to regulate adenylate cyclase activity. In cloned, differentiated rat hepatocytes (RL-PR-C), the plasma membrane acceptor for both cholera toxin-catalyzed and endogenous NAD^+-dependent ADP-ribosylation is a 55,000-dalton protein (5); endogenous ADP-ribosylation does not require cytosolic components (5). In view of the possi-

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bility that endogenous hepatocyte plasma membrane ribosyltransferase activity may be physiologically significant, we asked whether this activity might be modified by hormones which, like cholera toxin, stimulate adenylate cyclase in these cells. We provide here the first known report of an effect of a hormone, isoproterenol, on NAD^+-dependent ADP-ribosylation.

MATERIALS AND METHODS

[2,8-^{3}H-adenine] NAD^+ was obtained from New England Nuclear, cholera toxin from Schwarz-Mann, and l-isoproterenol and dl-propanolol from Sigma. The toxin was incubated prior to use for 10 minutes at 30° with 20 mM dithiolthreitol.

RL-PR-C hepatocytes (8) were maintained in Ham's F12 medium supplemented with 10% fetal bovine serum (9). A crude plasma membrane preparation was employed for assays of adenylate cyclase (10) and ADP-ribosyltransferase (5,11) activities. Thymidine (1 mM) was included in ADP-ribosyltransferase assays to inhibit nuclear poly(ADP-R) polymerase activity (12). The proteins of membrane extracts were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (5); under the conditions employed, neither DNA nor poly(ADP-R) enter the gel. Protein was determined by the method of Lowry et al. (13).

RESULTS AND DISCUSSION

Isoproterenol stimulated both the incorporation of ADP-ribose from NAD^+ into trichloroacetic acid-insoluble macromolecules and adenylate cyclase activity in hepatocyte membranes (Fig. 1); the effects of the hormone were concentration dependent. The activities, however, differed greatly in their sensitivity to isoproterenol: half-maximal activation of adenylate cyclase occurred at a concentration (1 nM) at which approximately 2% of the maximum stimulation of ADP-ribosylation occurred; half-maximal activation of ADP-ribosylation occurred with 1 μM isoproterenol. These relationships could be interpreted as suggesting that hormone activation of adenylate cyclase is tightly coupled to hormone-stimulated ADP-ribosylation.

The kinetics of both processes were quite similar, with the hormone effects being evident by about 2 minutes, and appearing to peak at about

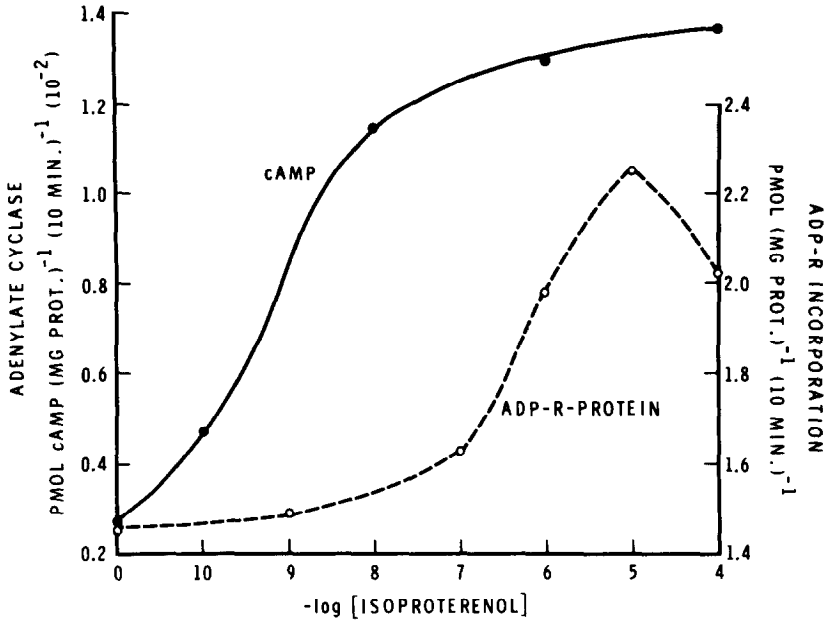

Fig. 1. Effect of isoproterenol on ADP-ribosylation and adenylate cyclase activity in hepatocyte membranes. ADP-ribosyltransferase (O) and adenylate cyclase (●) activities were measured in hepatocyte membranes for 10 minutes at 37° in the presence of the indicated concentrations of isoproterenol. The concentration of [2,8-^{3}H-adenine] NAD^{+} was 0.5 μM. The data are means of values from duplicate incubations in a representative experiment.

10 minutes (Fig. 2). In contrast, we observed previously (11) that cholera toxin activation of ADP-ribosylation substantially preceded its activation of adenylate cyclase in hepatocyte membranes. If a cause-and-effect relationship exists between both isoproterenol-stimulated activities, they must be very tightly coupled kinetically.

When hepatocyte membranes were incubated with the β-adrenergic antagonist, propanolol, prior to addition of isoproterenol, the agonist was without effect on ADP-ribosyltransferase activity (Table 1). This observation suggests that the effect of isoproterenol on ADP-ribosylation in hepatocyte membranes is, like its effect on adenylate cyclase, mediated through the β-adrenergic receptor. Furthermore, neither corticotropin nor thyrotropin, which stimulate adenylate cyclase in their

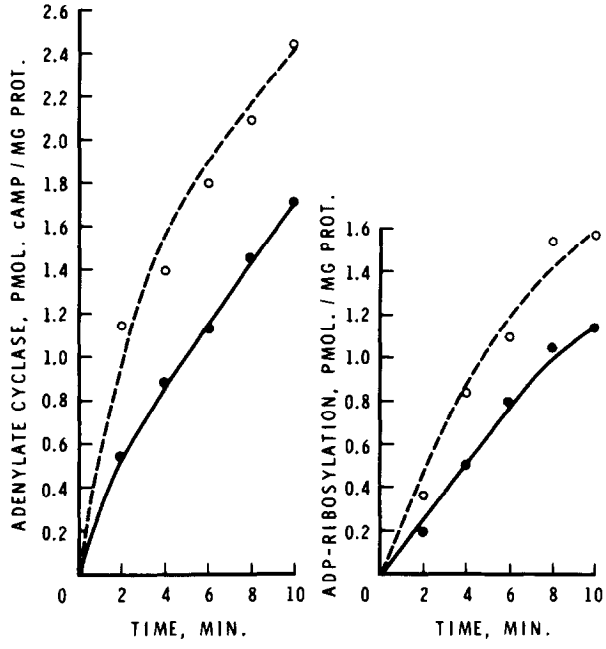

Fig. 2. Kinetics of isoproterenol-stimulated ADP-ribosylation and adenylate cyclase in hepatocyte membranes. Adenylate cyclase (left) and ADP-ribosyltransferase (right) progress curves were determined in the absence (●) or presence (○) of 10 μM isoproterenol at 37°. The concentration of [2,8-^{3}H-adenine] NAD^+ was 0.5 μM. The data are the means of values from duplicate incubations in a representative experiment.

target cells but not in RL-PR-C hepatocytes, influenced ADP-ribosylation in hepatocyte membranes (Table 1).

Hepatocyte membranes were incubated with [2,8-^{3}H-adenine] NAD^+ under ADP-ribosylation conditions, solubilized with sodium dodecyl sulfate, and the membrane proteins separated by polyacrylamide gel electrophoresis. As shown in Fig. 3, the acceptor protein for isoproterenol-enhanced ADP ribosylation appeared to be the same 55,000 dalton protein serving for both endogenous and cholera toxin-stimulated processes (5). No other acceptor proteins were evident.

It is believed that activation of adenylate cyclase by cholera toxin results from inhibition of GTP hydrolysis by an enzyme associated with the guanyl nucleotide regulatory protein (14). This action of

Table 1 Specificity of the Isoproterenol Effect on ADP-Ribosylation in Hepatocyte Membranes

Additions	ADP-Ribosylation pmol (mg prot)$^{-1}$ (10 min)$^{-1}$
Basal	0.70
Isoproterenol (10 μM)	1.17
Propanolol (1.0 mM)	0.77
Both	0.87
Corticotropin (1.0 μM)	0.62
Thyrotropin (1.0 μM)	0.62

Hepatocyte membranes were ADP-ribosylated with 0.5 μM [2,8-^{3}H-adenine] NAD$^+$ at 37° for 10 minutes in the presence of the indicated ligand. When employed, propanolol was incubated with membranes for 5 minutes at 37° prior to addition of NAD$^+$ and start of the 10 minute ADP-ribosyltransferase assay.

cholera toxin is apparently mediated by the ADP-ribosylation of the regulatory protein (1,2,15), which is 42,000 daltons in avian erythro

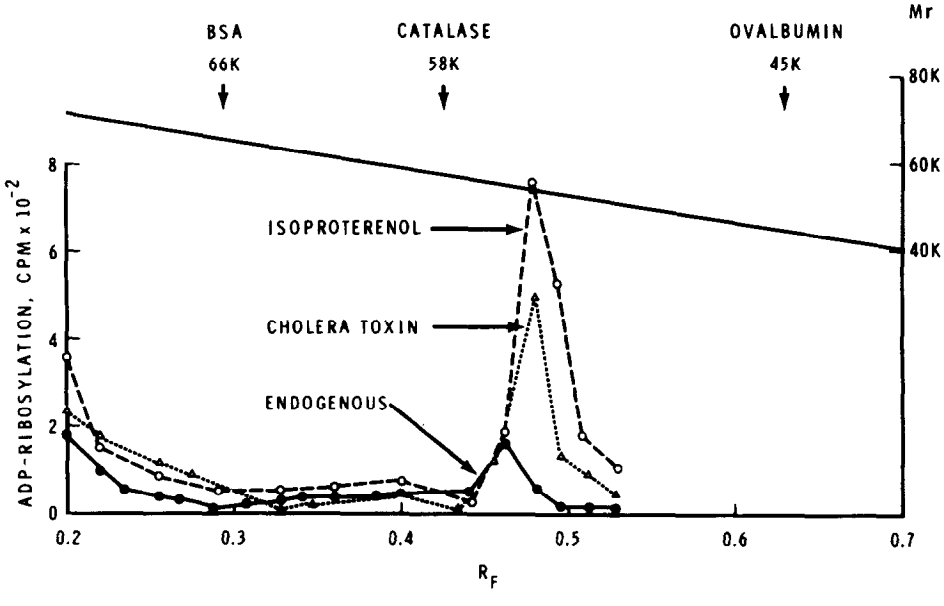

Fig. 3. Incorporation of [2,8-^{3}H-adenine] NAD$^+$ into a hepatocyte membrane protein. Hepatocyte membranes were ADP-ribosylated under basal conditions (●), in the presence of 5 μg/ml of cholera toxin (Δ) or 10 μM isoproterenol (O), then solubilized. Membrane proteins were separated by tube gel electrophoresis and radioactivity determined in 1 mm slices. The gel patterns shown are representative of results obtained in several experiments.

cyte ghosts (1), and apparently 42,000-45,000 and 52,000-54,000 daltons in S49 lymphoma cells (2,3) and 3T3 fibroblasts (4). Presumably, the single 55,000 dalton ADP-ribose acceptor protein of RL-PR-C hepatocytes is this regulatory subunit. Our observation, that isoproterenol stimulates ADP-ribosylation of this same protein seemingly by modulating the activity of a plasma membrane ADP-ribosyltransferase, suggests that catecholamine activation of adenylate cyclase in these cells depends upon this process. Although the evidence is preliminary and at best suggestive, nevertheless, this report raises intriguing possibilities concerning the molecular mechanism of catecholamine action, and the potential physiological significance of endogenous ADP-ribosylation.

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