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EVIDENCE FOR REVERSABILITY OF AGE-RELATED DECREASE IN HUMAN LYMPHOCYTE ADENYLATE CYCLASE ACTIVITY

J. Frederick Krall, Marianne Connelly, and Michael L. Tuck
Biochemical Pharmacology Laboratory of the
UCLA-SFVP Department of Medicine
VA Medical Center
Sepulveda, CA 91343

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SUMMARY: Age-related loss of adenylate cyclase responsiveness to guanyl nucleotide was demonstrated in lymphocytes freshly isolated from human subjects. Enzyme activity of cells from young (<40 years) and elderly (>65 years) subjects were markedly sensitive to inhibition by non-ionic detergents. When enzyme activity in the presence of guanyl nucleotide and low concentrations of Triton X-100 was determined in a mixture of cells from the young and aged donors, the activity was 40±17 percent (mean ± S.D.) greater than anticipated from the activity of the cells of the two age groups assayed separately. The detergent range which facilitated the enhanced enzyme activity was too low to extract the catalytic subunit of adenylate cyclase from the cells. These results further suggest that in man, changes distal to receptors contribute to diminished responsiveness of lymphocyte adenylate cyclase as a function of age. In addition, these age-related changes may be partially reversible by reconstitution with factors from cells from younger subjects.

Senescense has been associated with decreased target organ sensitivity to some hormones (1). The nature of the changes which result in diminished hormonal responsiveness need to be ellucidated, since reduced target organ sensitivity could contribute to the poorly understood aging process. Adenylate cyclase activity of lymphocytes from aged human subjects (>65 years) was lower in the presence of isoproterenol, guanyl nucleotide, NaF, as well as in the basal state, compared to enzyme activity in cells from younger (<40 years) subjects (2). The differences were not accounted for by age-dependent changes in T- and B-lymphocyte distribution, and suggested an age-related change in the enzyme distal to the β-adrenergic catecholamine receptor might account for hormone refractoriness associated with senescence. Decreases in either adenylate cyclase catalytic subunits

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or the guanyl nucleotide-requiring factors coupling receptors and catalytic subunits could have accounted for the lower activity.

Reconstitution of activated adenylate cyclase from its component parts has been achieved with enzyme from a variety of hormone target cells (3-5). Reconstituions are routinely acheived by combination of detergent extracts (6), but simple mixing of membranes from sources deficient in specific components has also been successful (7). Application of these reconstitution methods to the human lymphocyte suggest age-related changes in a human target cell might be reversible in the presence of factors in cells from younger subjects.

MATERIALS AND METHODS

Lymphocytes were isolated from blood collected in heparinized tubes from 27 healthy volunteers between the ages of 20 and 92 years of age according to the method described in (2). Cells were suspended in 0.05M Na^+-HEPES buffer (pH 7.6) with 0.001M EGTA and 10% dimethylsulfoxide at a density of 25 million per ml and used within 2 hours of preparation.

Lymphocyte adenylate cyclase activity was determined as the enzyme-dependent conversion of (α-^{32}P)-ATP to ^{32}P-cAMP followed by chromatographic purification of the radioactive product on Dowex and aluminum oxide columns (8). Details of the assay and properties of the enzyme are described in (2). Using these methods, enzyme activity in the presence of all additions was linear as a function of cell number until the concentration in the 0.15 ml reaction mixture exceeded 2 million cells. Linearity and sensitivity of the assay were unaffected by the low concentrations of Triton X-100 used in the assay.

RESULTS AND DISCUSSION

Guanyl nucleotide-dependent adenylate cyclase activity in lymphocytes decreased significantly ($p<0.02$) with the age of the blood donor (Figure 1), perhaps contributing to the age-dependent loss of hormone responsiveness as described in (2). Hormone-sensitive adenylate cyclase is a complex system composed of the receptor or recognition subunit, the catalytic subunit, and the guanylyl nucleotide requiring subunit(s) or coupling factor(s) which couple the other two components. All of the membrane components of the adenylate cyclase system can be extracted with non-ionic detergents, and

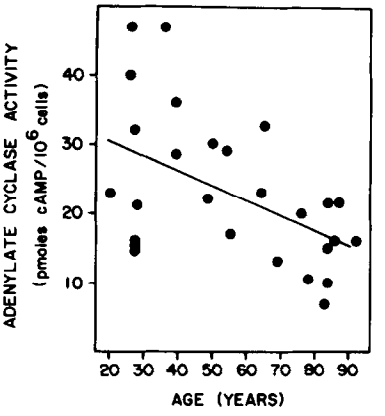


Figure 1. Effect of donor age on guanyl nucleotide-dependent adenylate cyclase activity. Lymphocyte enzyme activity in the presence of 100 μM Gpp(NH)p is plotted as a function of the age of the donor from whole blood cells were isolated. Each point is the mean of triplicate determinations on a preparation from a different donor. The correlation coefficient of the straight line, drawn using a least squares program, was 0.51 ($p<0.02$, $n=27$).

partially as well as highly purified coupling factors have been used to reconstitute guanylyl nucleotide (and hormone) responsiveness of S49 lymphoma cells (6,9,10).

Both Triton X-100 and Lubrol PX inhibited the lymphocyte enzyme at low concentrations (Figure 2, inset). These inhibitory effects were enzyme-specific, since adenylate cyclase activity of rat uterine smooth muscle was far more resistant to detergent-inhibition using the same assay conditions (Figure 2). Appreciable extraction of the catalytic subunit, as reflected by an increase in enzyme activity present in the 20,000xg supernatant of detergent-treated cells, only occurred at Triton X-100 concentrations >0.006% (Figure 3). When the detergent-sensitivity of adenylate cyclase was compared in cells from young or aged subjects, it was the same (not shown) with a marked exception. Enzyme activity was consistently enhanced in the presence of detergent and Gpp(NH)p when cells from aged subjects were mixed with those from young subjects then assayed (Figure 4).

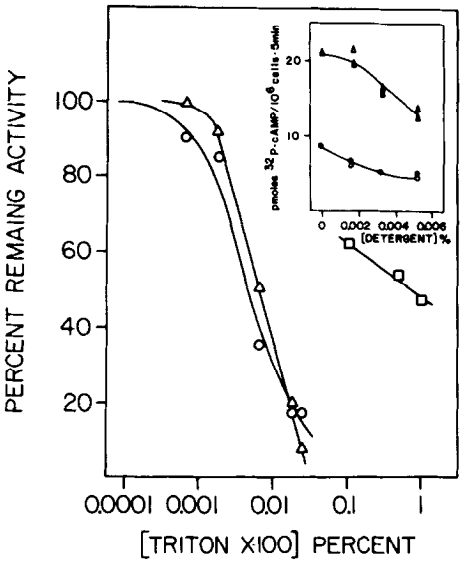
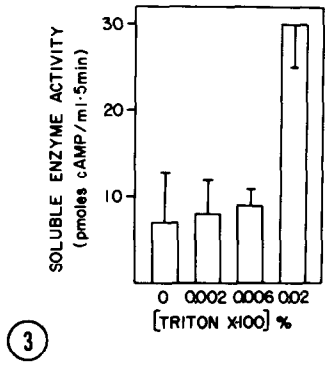


Figure 2. Detergent sensitivity of human lymphocyte adenylate cyclase. In the inset, activity in the presence (Δ,▲) and absence (o,●,basal activity) of 10 μM Gpp(NH)p was determined at the indicated concentrations of either Triton X-100 (open symbols) or Lubrol PX (closed symbols). In the principal figure, human lymphocyte enzyme activity determined in the presence (Δ) or absence (o) of 10 μM Gpp(NH)p is compared with rat uterine smooth muscle enzyme activity in the presence of 100 μM Gpp(NH)p (🖳) as a function of Triton X-100 concentration. Results are the mean of duplicate determinations. The rat enzyme was prepared as described in (13).

In four different mixing experiments using cells from separate donors, the activity of "young" and "aged" cells assayed together in the presence of low detergent concentrations exceeded by 40 ± 17 percent (mean ± S.D.) the activity expected from assaying them separately under identical conditions and subsequently adding the activities. The effect required detergent, and occurred at Triton X-100 concentrations up to 0.01%.

Enhanced activity of the mixed cell enzyme system was not due to increased detergent binding by the cells, since these low detergent concentrations had no effect on the linearity of the enzyme assay in excess of 2 million cells per assay tube (not shown). Rather, these would be the anticipated results where diffusable factors from the "young" cells were able to reconstitute the lower enzyme activity of the "aged" cells



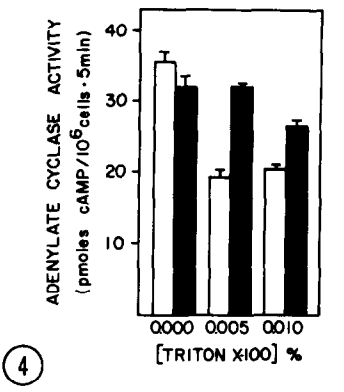


Figure 3. Solubilization of lymphocyte adenylate cyclase with Triton X-100. Freshly isolated cells were incubated at room temperature in 0.05M HEPES, pH 7.6; 0.001M EGTA; 10% DMSO; 10 μM Gpp(NH)p, and the indicated concentrations of Triton X-100 at a density of 10 million cells per ml. After 15 min the suspensions were chilled to 4°C and centrifuged for 20 min at 20,000xg. The supernatants (0.04ml) were assayed in triplicate for enzyme activity in the presence of 10 μM Gpp(NH)p. Results are the mean ± S.D. of the three determinations.

Figure 4. Effect of mixing lymphocytes from young and old donors on adenylate cyclase activity in the presence of Gpp(NH)p. Adenylate cyclase activity in the presence of 10 μM Gpp(NH)p and the indicated concentrations of detergent was determined using: 0.5 million cells from a young (<40 yrs) donor; 0.5 million cells from an aged (>65 yrs) donor; and 0.5 million cells from the aged donor in the presence of 0.5 million cells from the young donor. Enzyme activity of the cells from the two age groups measured separately were added together (open bars) for comparison with activity when mixed together (closed bars). The results, the mean ± S.D. of triplicate determinations of a single experiment, are representative of 4 separate experiments using cells from different donors over the same detergent concentration range. In all the experiments, enzyme activity was 40 ± 17 percent (mean ± S.D.) greater when the cells from the two donor age groups were mixed together and assayed than when assayed separately and the activities subsequently added. Activity of the different cell preparations in the presence of 10 μM Gpp(NH)p was: young, 33.7±4.0 pmole cAMP/10^6 cells; aged, 18.5±3.0 pmole cAMP/10^6 cells.

in a detergent-dependent manner. The low detergent concentration over which the effect occurred compared with that needed to extract enzyme into the 20,000xg supernatant suggests the putative factors were not adenylate cyclase catalytic subunits. Similarly, membrane associated guanylyl nucleotide-requiring coupling factors are reported resistant to the relatively mild treatments that extract extrinsic membrane proteins and appear associated with the lipid bilayer by relatively strong hydrophobic interactions (11). The enzyme activity in the mixed cell system was

responsive to Gpp(NH)p, however, so guanylyl nucleotide-requiring factors cannot be excluded. Paradoxically, transfer of guanyl nucleotide-requiring coupling factor(s) from human erythrocytes to S49 lymphoma cells deficient in the factors occurs in the absence of detergent altogether (7).

In addition, substantial concentrations of soluble cytoplasmic proteins with coupling factor properties but with a poorly understood relation to the membrane form of the protein(s) have been described in rat liver, heart, and skeletal muscle, and in dog and rabbit liver (12). Either of these little understood transfer mechanisms may have been facilitated by the low detergent concentration range in which enzyme activity was enhanced in mixtures of cells from young and aged subjects.

Age-related decreases in human lymphocyte adenylate cyclase sensitivity to guanyl nucleotide as well as isoproterenol (2) cannot be explained solely by decreased β-adrenergic receptor number and indicate alterations in the number or state of guanylyl nucleotide-coupling factors or catalytic subunits, or the interactions between them. Our results suggest but do not prove that diminished adenylate cyclase responsiveness of lymphocytes from aged subjects are partially reversible, perhaps by reconstitution of the coupling factors which activate the adenylate cyclase catalytic subunit.

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