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REDUCED SENSITIVITY TO CATECHOLAMINE IN WERNER'S SYNDROME FIBROBLASTS

Shiro Bannai*, Naomichi Okamura*, Tetsuro Ishii*, Yoshiki Sugita*,
Chieko Bannai**, and Kamejiro Yamashita**

*Institute of Basic Medical Science, and **Institute of Clinical Medicine,
University of Tsukuba, Sakura-mura, Ibaraki 305, JAPAN

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SUMMARY: The β-adrenergic receptor-coupled adenylate cyclase system has been investigated in normal and Werner's syndrome fibroblasts. The basal levels of cAMP in Werner and normal control cells were similar, whereas the isoproterenol-induced increase in cAMP levels was far less for Werner cells than for control cells. In the broken cell preparations isoproterenol stimulated the adenylate cyclase of only control cells, not of Werner cells, although NaF or prostaglandin E_1 stimulated the enzyme of both cells to the same extent. The β-adrenergic receptor concentrations analyzed with hydrophilic radioligand were nearly equal in Werner and in control cells. A reduction of functional activity of the β-adrenergic receptor in Werner cells is thus suggested. © 1987 Academic Press, Inc.

Werner's syndrome (WS) is a rare condition of autosomal-recessive inheritance, showing some features of accelerated ageing (1,2). Although it is implied that WS is a partial copy rather than a full model of accelerated ageing, the identification of its molecular defect could help unravel the mystery of the ageing process. Cultured fibroblasts from patients with WS show very limited proliferative capacity in vitro when compared with normal controls from individuals of the same chronological age (3), indicating that the genetic defect manifests in the cultured cells. Since hormonal responsiveness is a candidate for alteration with increasing age (4), we have investigated the β-adrenergic receptor-coupled adenylate cyclase system in WS fibroblasts.

MATERIALS AND METHODS

Two strains of WS skin fibroblast, WS1 and WS2, were derived from skin biopsies of unrelated 56-year-old man and 25-year-old woman,

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respectively. Strain N1 and N2 were derived from the skin of healthy volunteers. They were grown in Eagle's minimum essential medium supplemented with 15% fetal calf serum. Cultures of normal and WS cells were passaged at a split ratio of 1:2. N1 and N2 cells were able to grow until about 35-40 transfers, whereas both WS1 and WS2 cells ceased to grow after about 10 transfers.

For measurement of cAMP levels, cells were washed three times with phosphate-buffered saline and extracted with 5% trichloroacetic acid. The acid extract was treated with diethyl ether and an aliquot of the aqueous solution was assayed for cAMP using radioimmunoassay kit (New England Nuclear).

For the assay of adenylate cyclase, cells were homogenized in ice-cold 5 mM Tris-0.25 M sucrose-0.2 mM $MgCl_2$, pH 7.5, and the homogenate was centrifuged at 20,000 x g for 15 min. The precipitate was used for the enzyme assay as described previously (5).

For the assay of β-receptors, cells were washed three times with Earle's balanced salt solution supplemented with 10 mM HEPES and incubated at 37°C in the same solution containing [^{3}H]CGP-12177 (Amersham) with and without 10 μM DL-propranolol. After 30 min the cells were washed four times with cold phosphate-buffered saline. The total washing time was standardized to 10 min. The cells were then lysed with 0.5 N NaOH and the aliquots were used for the determination of radioactivity and protein.

RESULTS

Cultured human fibroblasts respond to catecholamines with increased cAMP levels and the increase depends on the time after seeding of the cells and the cell density (6-8). We have investigated the isoproterenol effect on normal and WS fibroblasts. The magnitude of the effect on both cells reached a maximum around day 2-3 after start of culture and then declined. Therefore, in the experiment shown in Fig. 1, cells were plated at various densities and after 3 days the effect of isoproterenol was measured. The cAMP contents of normal and WS cells incubated without isoproterenol were 3-10 pmol/mg cell protein and no significant difference was found between normal and WS cells. Isoproterenol caused a great increase in cAMP in normal fibroblasts. The isoproterenol effect appeared to be lessened at high cell densities, although it varied considerably from experiment to experiment. In contrast, WS cells responded poorly to isoproterenol. Response of strain WS2 was greatly affected by cell density. Response of strain WS1 was even poorer than that of WS2 and the changes associated with cell density were less marked. Fig. 1 shows also the isoproterenol effect on normal "senescent" cells. These cells mimic WS cells in

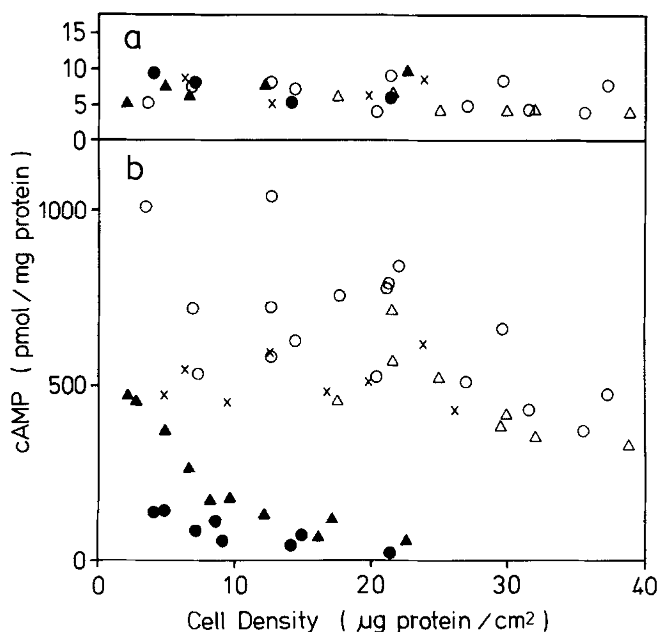

Fig. 1. Effect of isoproterenol on cAMP levels of normal and WS cells grown to varied densities. Cells, which had been seeded at various densities in 35 mm diameter dishes 3 days before, were washed three times with Eagle's basal medium and incubated at 37°C for 5 min in the same medium (1 ml) not containing (a) or containing (b) 10 µM (±)-isoproterenol, and cAMP levels in the cells were measured. ○, N1 (normal) cells, less than 25 passages in culture; x, N1 cells, more than 35 passages in culture; △, N2 (normal) cells, less than 25 passages in culture; ●, WS1 cells; ▲, WS2 cells. Each point represents mean value for two dishes. Data for at least three separate experiments on each cell strain are presented.

that they grow very slowly and are enlarged and somewhat randomly orientated in monolayer culture. However, the normal senescent cells responded well to isoproterenol and it was hard to find significant difference between the responses of young and senescent cells.

To investigate the mechanisms involved in changes in hormonal responsiveness, an analysis of the adenylate cyclase system was carried out in broken cell preparations. As shown in Fig. 2, the enzyme activities of the particulate fractions of normal cells, both young and senescent in culture, were stimulated by isoproterenol, whereas those of WS cells were not stimulated significantly. Basal activities of adenylate cyclase of normal and WS cells were similar. Prostaglandin E_1- or NaF-stimulated adenylate cyclase activities of normal and WS

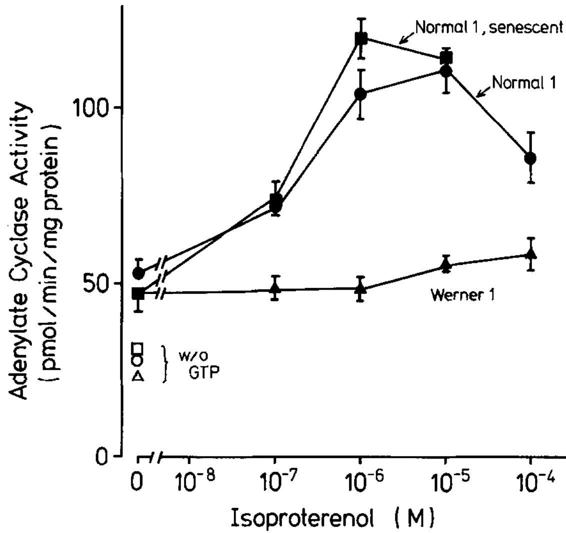

Fig. 2. Effect of isoproterenol on the adenylate cyclase of normal and WS cells. Cells were seeded and cultured in roller bottles, and after 3-4 days, at the density of 10-25 μg protein/cm^2, they were collected and assayed for adenylate cyclase activity. ●, N1 cells, less than 25 passages in culture; ■, N1 cells, more than 35 passages in culture; ▲, WS1 cells. Open symbols show the activities in the absence of GTP in the assay mixture. Each point represents mean ± s.e. of triplicate assays. The data shown are typical of three similar experiments.

cells were also measured; in the presence of 10^{-5} M prostaglandin E_1 or of 10 mM NaF the enzyme activities of both cells equally increased by about 8-fold and 7-fold, respectively.

The different response of intact cells or of broken cell preparations to isoproterenol could be explained by alterations in cell surface receptors. Therefore, the concentration of β-adrenergic receptor was assessed in normal and WS cells using [^{3}H]CGP-12177. This compound is a hydrophilic β-adrenergic receptor radioligand suitable for receptor analysis in intact cells (9). Data on the binding of CGP, present in excess, to normal and WS cells at various population densities are shown in Fig. 3. Unexpectedly, there was no significant difference between normal and WS cells in the capacity of CGP binding. The amount of CGP bound to the cells increased at low cell densities, indicating an increase in the receptor concentration on the cell surface. From Scatchard analysis of the binding data (not

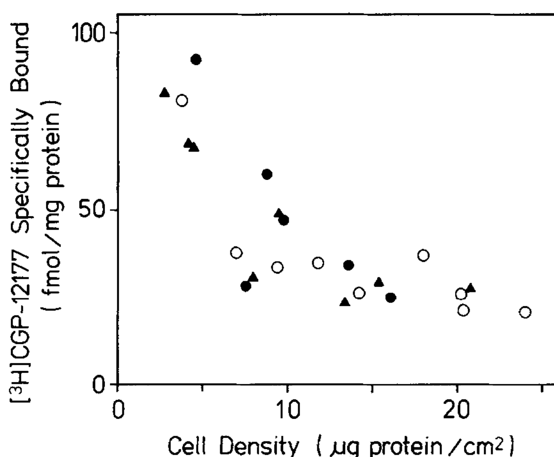

Fig. 3. Binding of [^{3}H]CGP-12177 to normal and WS cells grown to varied densities. Cells, which had been seeded at various densities in 60 mm diameter dishes 3 days before, were incubated with 10 nM [^{3}H]CGP-12177. For each point in the figure four dishes were used; two for the bindings in the absence of DL-propranolol and two for those in the presence of DL-propranolol. Specific binding was defined as the amount of [^{3}H]CGP-12177 bound in the absence of competing ligand minus the amount bound in the presence of 10 μM DL-propranolol. ○, N1 cells, less than 25 passages in culture; ●, WS1 clels; ▲, WS2 cells.

shown), K_d values of 0.9-1 nM for CGP were obtained in both WS and normal cells. The numbers of binding sites in normal and WS cells at relatively high cell densities were almost the same if compared on the basis of cell protein. Because WS cells are larger than normal cells, the numbers of binding sites per cell were calculated to be 6500 in normal cells and 12000 in WS cells. Preliminary experiments using [^{125}I]iodohydroxybenzylpindolol also showed that the receptor concentrations were similar in normal and WS cells.

DISCUSSION

In studies with human fibroblasts the magnitude of the increase in cAMP produced by catecholamines was inversely related to cell density (6-8). Changes in the number of β-adrenergic receptors per cell would appear to account for the divergent cellular responses (10). In the present study, while WS cells responded very poorly to isoproterenol both in intact cells and in broken cell preparations,

the apparent density of β-adrenergic receptor was not reduced. It is possible that WS cells have an unusually high activity of phophodiesterase that may lower the cAMP levels. However, this possibility was excluded by examining the isoproterenol effect in the presence of isobutyl methylxanthine, an inhibitor of phosphodiesterase. The degree of potentiation of the isoproterenol effect by this compound was similar in WS cells and in normal cells.

Adenylate cyclase activity of WS cells was greatly stimulated by prostaglandin E_1 or by NaF to the same extent as that of normal cells, indicating that the catalytic unit and the regulatory unit (guanine nucleotide-binding protein) of the adenylate cyclase system in WS cells are normally functioning. Thus the most probable explanation for the declined sensitivity of WS cells to catecholamines is a reduction in functional activity of the β-adrenergic receptor. However, the nature of human β-adrenergic receptors is largely unknown and a novel component is being found (11). In human fibroblasts catecholamines produce their largest effect on sparsely cultured cells which are less typically fibroblastic in morphology (8). Responsiveness to catecholamines seems to be relevant to specific functional differentiation or maturation, and its loss in WS cells may result in an impairment in the development of connective tissue in this disorder.

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