Scanning Microscopy

Volume 2 | Number 4 Article 31

7-31-1988

Effect of X-Irradiation on Adenylate Cyclase Activity and Cyclic AMP Content of Primary Human Fibroblasts

Z. Somosy
"Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

A. Takáts
"Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

Tamara Kubasova
"Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

F. Sudár
National Institute of Oncology

G. J. Köteles
"Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

Follow this and additional works at: https://digitalcommons.usu.edu/microscopy

 Part of the Life Sciences Commons

Recommended Citation

Somosy, Z.; Takáts, A.; Kubasova, Tamara; Sudár, F.; and Köteles, G. J. (1988) "Effect of X-Irradiation on Adenylate Cyclase Activity and Cyclic AMP Content of Primary Human Fibroblasts," *Scanning Microscopy*: Vol. 2 : No. 4 , Article 31.
Available at: https://digitalcommons.usu.edu/microscopy/vol2/iss4/31

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

Scanning Microscopy, Vol. 2, No. 4, 1988 (Pages 2165-2172) 0891-7035/88$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

EFFECT OF X-IRRADIATION ON ADENYLATE CYCLASE ACTIVITY AND CYCLIC AMP CONTENT OF PRIMARY HUMAN FIBROBLASTS

Z.Somosy*, A.Takáts, Tamara Kubasova, F.Sudár+ and G.J. Köteles

"Frédéric Joliot-Curie"
National Research Institute
for Radiobiology and Radiohygiene
H-1775, Budapest, POBox 101
Hungary

+National Institute of Oncology
Budapest, Hungary

(Received for publication February 22, 1988, and in revised form July 31, 1988)

Abstract

Ionizing radiation provokes an increase of the cAMP level in several organs and body fluids. After reviewing the relevant literature we present the results of our own experiments on primary human fibroblasts. X-irradiation at doses of 0.5 and 2.5 Gy in vitro evoked a rapid and reversible increase of adenylate cyclase enzyme activity. A significant increase in cAMP level of these cells was also observed.

Adenylate cyclase was usually localized basolaterally on the surface of unirradiated cells, while irradiation resulted in a modification of distribution, i.e., the enzyme activity also appeared in apical localization.

Key words: X-irradiation, adenylate cyclase activity and distribution, cyclic AMP content, electron microscopy, human fibroblasts.

*Address for correspondence:
Z.Somosy, "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, POBox 101, Hungary Phone No.: 36 1 730026

Introduction

The cAMP contents in tissues, organs and certain body fluids are modified, usually increased, by ionizing radiation. A few examples are listed on Table 1.

As cAMP is a second messenger in signal transfer, it plays an important role in the interaction of the cell and its environment as well as in the regulation of growth and differentiation (29, 30,35,43). Similar to these environmental or physiological stimuli, a change of its concentration due to the effect of X-irradiation may lead to alterations of cellular functions. Thus a change in cAMP level may be one of the reasons for the radiation-induced delay of mitosis (8,9) and may contribute to the radiation effect on the lysosomal system of the cells (45,46). It is also known, however, that the increase of cAMP level induced experimentally by phosphodiesterase inhibitors exerts certain radioprotective effects (2,7,13,33). Moreover, the elevation of cAMP concentration contributes to the protective action of some radioprotectors like cysteamine, WR-2721 and 2-mercaptoethylamine (6,7,41,46).

The increase of cAMP levels after in vivo irradiation may be the consequence of either an increase of adenylate cyclase activity or the inhibition of phosphodiesterase action.

Activation of adenylate cyclase has been described in murine spleen after in vivo and in isolated liver cell membranes (23,24) after in vitro X-irradiation (41). No data are available about the in vitro induced changes in cAMP level and adenylate cyclase activity in tissue cultures which are frequently used models in the study of different radiation effects. Our earlier investigations suggested that certain membrane related phenomena of human fibroblasts are rather sensitive to ionizing radiation (20,21, 22,40).

Despite the multitude of information available about the metabolism of cAMP

Table 1.

Effects of ionizing irradiation on cyclic AMP level or adenylate cyclase activity[+]

Test object		Type of irradiation	Dose, Gy	Changes in cAMP level or adenylate cyclase activity[+]	References
In vivo whole body irradiations					
Rat	liver	^{60}Co-gamma	10	increase (max. 3 h)	(46)
			10	increase (max. 4 h)	(36)
			7.5	increase (max.48 h)	(31)
			5	increase (max.48 h)	(31)
			4	increase (max. 4 h)	(36)
	spleen		10	decrease	(36)
			10	increase (max. 6.72 h)	(46)
			4	decrease	(36)
	intestinal mucosa		7.5	slight increase (max. 5 days)	(31)
			5	increase (max.10 days)	(31)
	heart		10	(decrease 4.72 h, increase 24 h)	(36)
			4	(decrease 24 h, increase 72 h)	(36)
	brain		7.5	increase (max.10 days)	(31)
			5	increase (max.10 days)	(31)
	blood plasma		10	decrease	(31)
			4	increase (max. 24 h)	(31)
Mouse	spleen	X-ray	8	decrease	(41)
			4	increase (max. 15 min)	(41)
			2	increase (max. 3 h)	(41)
	brain	^{60}Co-gamma	7.5	increase (max.10 days)	(31)
			5	increase (max.10 days)	(31)
Monkey	brain (local irradiation	high energy electron	100	decrease	(14)
			9	decrease	(6)
In vitro irradiation					
Isolated rat liver cell membranes		^{60}Co-gamma	10	does not change	(23,24)
			2.5	increase (max.10-30 min)	(23,24)
Physarum (fungus)			0.5	increase (max.10-30 min)	(23,24)
			24	increase (10 min)	(8)
			10	increase	(8)

in fibroblasts (1,42,44), the relevant radiobiological data are very scarce. Therefore, in our present work the radiation-induced changes in cAMP level and adenylate cyclase activity were also examined in this cell type. Efforts were made to study the radiation induced alterations with electron microscopic cytochemical reactions and by parallel biochemical measurements. Special attention was paid to the regional localization of adenylate cyclase activity in fibroblasts as according to some studies (10,27,28, 38) it is polarized with basolateral predominance in various cell types.
Until now, however, no data are available concerning the localization of the enzyme on fibroblast surfaces.

Materials and Methods

Cell culture

Human fibroblasts obtained from skin biopsies were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. For the experiments three-day cultures were used. Embryo fibroblasts were collected after slight trypsinization of human embryo tissues obtained from healthy pregnancies interrupted at 2-3 months. Details of cell isolation and culturing were described earlier (40).

Irradiation

X-irradiation was performed with THX-250 machine. Conditions: 200 kV, half value layer (h.v.l.) 1.0 mm Cu, source-surface distance (S.S.D.) 90 cm, dose-rate 0.317 $Gy_{water}.min^{-1}$. The exposures were measured by a calibrated ionization chamber (Farmer dose meter type 250 3.0.6.cm^3 ionization chamber). For the calculation of absorbed dose in water from the exposure, the rad/R conversion factor and the value of h.v.l. were used (16,17). The irradiation of cells was performed in tissue culture medium without calf serum. After irradiation the medium was replaced by the original one.

Electron microscopic cytochemical demonstration of adenylate cyclase

For the cytochemical localization of adenylate cyclase activity the 5' adenylylimidodiphosphate (AMP-PNP) method was

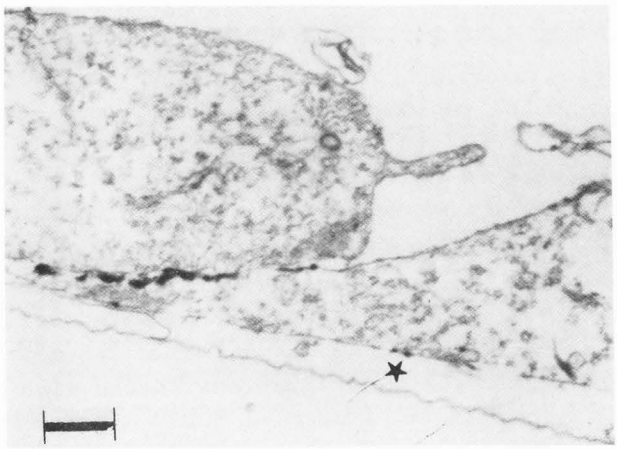


Figure 1. Adenylate cyclase activity visualized by histochemical reaction on primary human embryonic fibroblasts. The enzyme activity is localized on the basal surface (✱), and cell-to-cell contacts. Bar=0.5 /um.

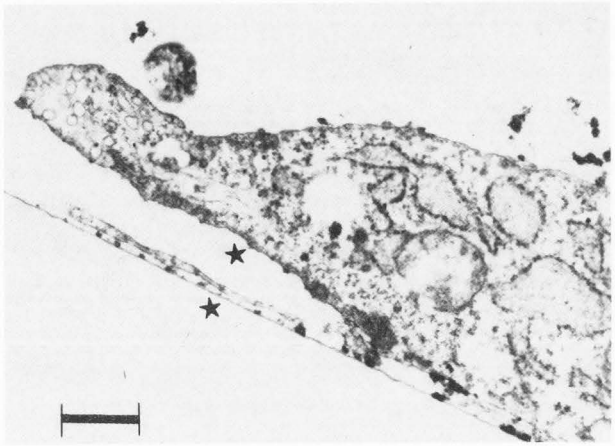


Figure 2. Adenylate cyclase activity on human skin fibroblast. The localization of the enzyme is similar to that observed on embryonic fibroblasts. It appears on the basal membrane regions. ✱ =basal surface. Bar=0.5 /um.

used (42,47). Initially, the cells were fixed for 30 min with 0.05 per cent glutaraldehyde in phosphate buffer. After rinsing the cells in 80 mM Tris maleate buffer (pH 7.4) the cells were incubated in adenylate cyclase assay medium consisting of 80 mM Tris maleate (pH 7.4), 10 mM $MgSO_4$ 2 mM theophyllin, 6 per cent sucrose and 0.6 mM AMP-PNP (Sigma).
Then the cells were rinsed again. Control experiments included the incubation of cells without AMP-PNP. The adenylate cyclase activity was investigated after X-ray treatment 10-30-60 mins with 0.5, 2.5 and 8 Gy doses. Three experiments were performed in each group. With the aim of semi-quantitative evaluation of adenylate cyclase activity we determined the extent of the whole surface covered by cytochemical reaction products at magnification 34500x. The covered fraction was expressed as a percentage of the whole surface. Twenty-five photographs were analysed.

After the cytochemical reaction the cells were fixed on coverslips with 2.5 percent glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at 4°C for 1 h. After washing with the same buffer the cells were further fixed with 1 per cent OsO_4 at 4°C for 1 h. The material was dehydrated in graded acetone and embedded in Durcupan AC (Fluka). After 24 h polymerization at 56°C the specimens were immersed in liquid nitrogen for 10-15 sec. This resulted in a clear separation of the Durcupan block from the coverslip. Ultrathin sections were cut with a glass knife on an LKB ultramicrotome in orientations both perpendicular to and parallel with the plane of the cell culture. The sections were examined with a JEM 6C type transmission electron microscope.

Estimation of cAMP level by radioimmunoassay (RIA)

The embryo fibroblast culture was stirred gently and centrifuged. The supernatant was decanted and cold 10 per cent TCA (trichloroacetic acid) was added to pellet. Then the pellet was homogenized and the precipitated proteins were removed by centrifugation. The supernatant was extracted with water saturated ether (ethylether) to remove TCA. Ether extraction was repeated three times. Ether contamination was removed by N_2 bubbling of the solution. Cyclic AMP content of samples was determined by cAMP-RIA according to Brooker et al (5). Sc-cAMP-TME (2'-O-monosuccinyl-adenosine 3' : 5' Cyclic Monosphosphate tyrosylmethylester) (Sigma) was labelled in our Institute with ^{125}I. The cAMP-antiserum was kindly supplied by Dr. A.Seregi (Institute of Experimental Medicine of Hungarian Academy of Sciences, Budapest).

Results

The adenylate cyclase activity visualized by the electron microscopic cytochemical reaction proved to be polarized on the plasma membranes of human embryo and skin fibroblasts; i.e., the enzyme activity appeared at the basolateral regions of the cells (Figs.1 and 2).
The enzyme activity and localization at the cell surface changed 10 and 30 minutes after X-irradiation with 0.5 or 2.5 Gy (Figs 3,4,5,6).

The amount of lead precipitate suggested elevated enzyme activity basally (Fig.3) and at the cell-to-cell contacts (Fig.4). The polarity of enzyme distribution was often diminished or eliminated by X-irradiation. Thus, after irradiation, the enzyme activity could frequently be visualized on the entire surface of the cell (Figs. 5 and 6). For comparison a much higher dose was also studied. The intensity of the cytochemical reaction, i.e., eznyme activity, was similar to that in the control cells after 8 Gy, and its polarized localization was evident (Fig.7). One hour after low dose X-irradiation the enzyme activity and localization became similar to the control (Fig.8).
In some cases the enzyme activity appeared in cell organelles like the granular endoplasmic reticulum, mitochondria, and nuclear membrane (Fig. 3,5,8).
In sections prepared perpendicularly to the plane of cell culture, the coverage of plasma membrane by reaction products was assessed. The data shown in Table 2. give evidence of semiquantitative changes of the amount of AC reaction products after X-irradiation.

Parallel with the changes of adenylate cyclase activity, studies were performed to determine the cAMP content of the cells. According to our preliminary data ionizing radiation induces readily measurable alterations of cAMP in cultured cells. The cAMP content of unirradiated cells proved to be 6.11 pmols per 10^6 cells. This value is in good agreement with the data obtained for mouse fibroblasts (44). Ten minutes after X-irradiation with 0.5 and 2.5 Gy the cAMP content increased considerably as the measured values were 35.0 and 27.8 pmols per 10^6 cells, respectively. One hour after low dose X-irradiations the cAMP content became similar to enzyme activity of the control. The changes of cAMP content after X-irradiation with 0.5 Gy were summarized on the Fig.9.

Discussion

Despite the interest of several laboratories in studies of the radiation-induced alteration of cAMP levels, investigations on cultured cells are rare. This situation gave an impetus to our studies as presented.
Our cytochemical data suggest that the elevation of cAMP concentrations following irradiation is due to an increase in adenylate cyclase activity. A similar rapid increase in adenylate cyclase activity has been described in the spleen of mice submitted to X-ray irradiation in vivo (41) and in isolated liver cell membranes after gamma irradiation (22,23). Adenylate cyclase is an intrinsic membrane enzyme consisting of several basic units, and changes in its micro-environment, or changes of forces acting on membrane constituents, could be consistent with alterations in membrane fluidity (11,18,37). Since an increase of membrane fluidity is accompanied by an increase in

Figure 3. Adenylate cyclase activity on human embryonic fibroblasts 10 minutes after X-irradiation with 0.5 Gy. The activity of enzyme increased on the basal membrane regions. The endoplasmic reticulum (⟶) is labelled by reaction products, too. ✶ =basal surface. Bar=0.5 /um.

Figure 4. Adenylate cyclase activity on primary human fibroblast 10 minutes after X-irradiation with 0.5 Gy. The increase of enzyme activity often can be seen at the cell contacts (➡) and elevated cell edges (➤). ✶ =basal surface. Bar=0.5 /um.

Figure 5. The X-irradiation induced high enzyme activity (0.5 Gy, 30 min) sometimes can be observed on the whole cell surface. ✶ =basal surface. Bar=0.5 /um.

Figure 6. The adenylate cyclase activity is changed by 2.5 Gy dose of X-irradiation in 10 mins. The enzyme activity can be seen both on the basal ✶, and apical ✶ membrane regions. Bar=0.5µm

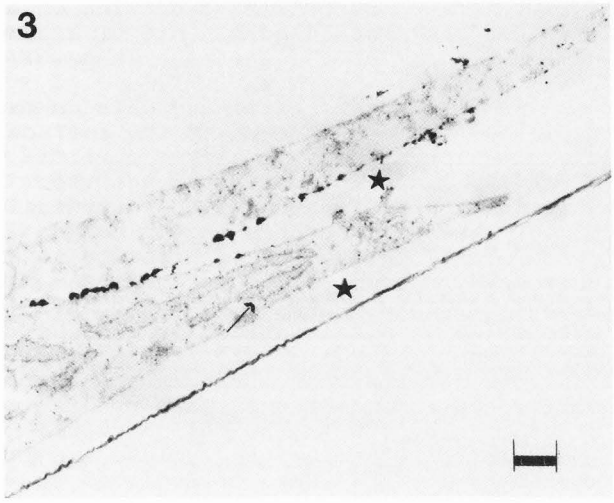
Figure 7. Adenylate cyclase activity on fibroblast 30 minutes after X-irradiation with 8 Gy. The enzyme activity did not change. Bar=0.5 /um.

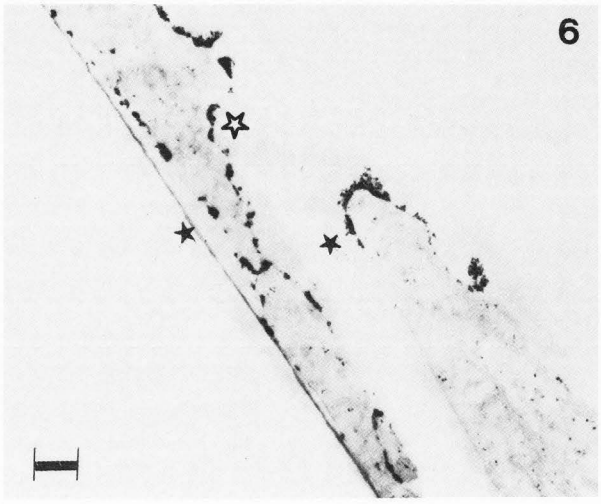
Figure 8. Adenylate cyclase activity on primary human embryonic fibroblasts 60 minutes after X-irradiation with 0.5 Gy. The activity and localization of enzyme became similar to the control. Enzyme activity on the endoplasmatic reticulum can be seen (⟶). ✶ =basal region. ➤ = contact region. Bar=0.5 /um.

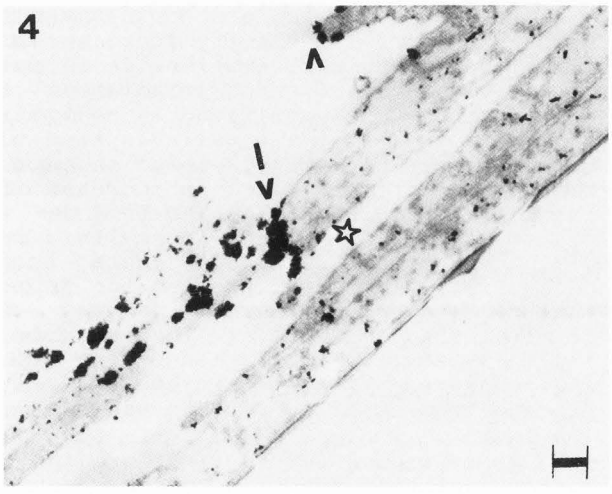
Table 2.

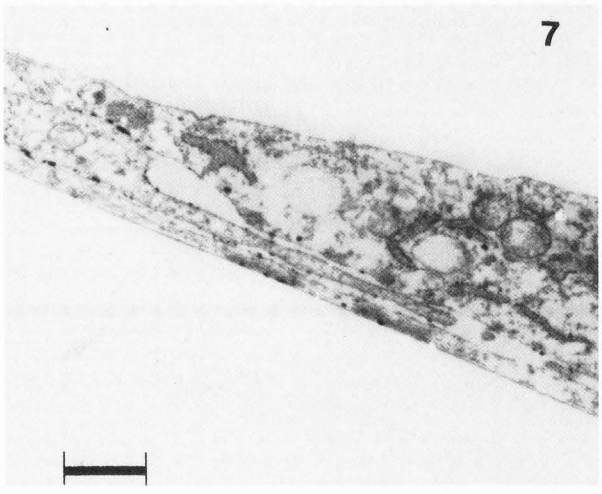
Change of AC reaction products covering the human fibroblast surfaces after X-irradiation

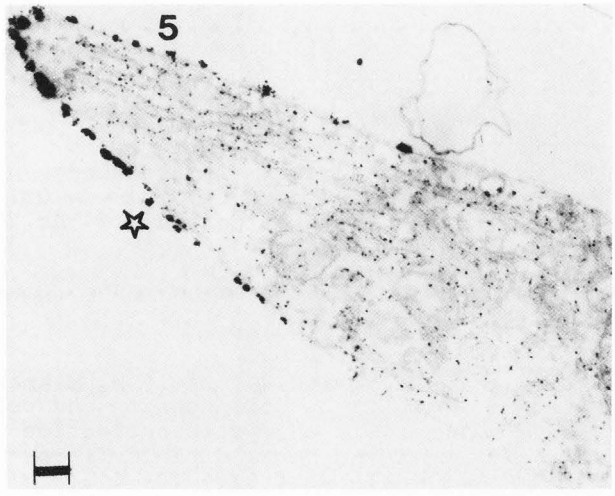
Dose (Gy)	Time after irradiation	AC reaction products covering the total surface in per cent
control	-	3.5 ± 2.8
0.5	10	10.3 ± 7.7
	30	8.9 ± 7.0
	60	2.6 ± 2.3
2.5	10	11.7 ± 9.9
	30	10.4 ± 8.5
	60	3.3 ± 2.5
8	10	4.3 ± 3.4
	30	3.9 ± 3.2
	60	4.1 ± 2.9

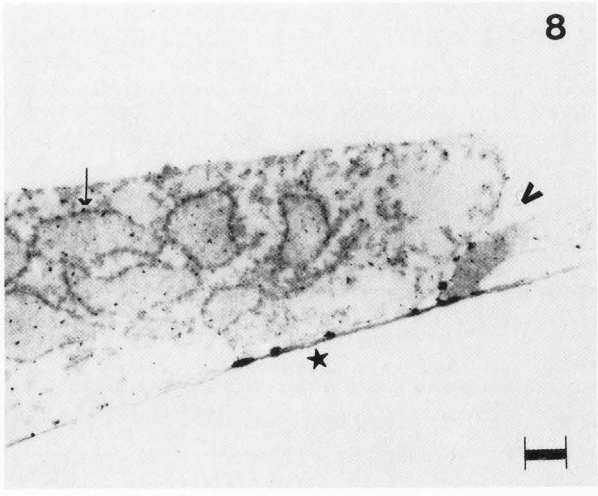

3


6


4


7


5


8

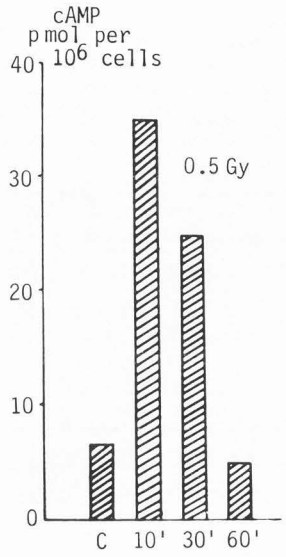


Figure 9. The changes of cAMP content after X-irradiation with 0.5 Gy.

the basal activity of adenylate cyclase (11,37), radiation-induced increase of enzyme activity is also likely to be connected with enhanced membrane fluidity, known to be elicited by irradiation (34). Our observation that sublethal doses do not evoke any increase in enzyme activity and cAMP level was also observed by other authors, (23,41). This is in agreement with the view that high radiation doses evoke an opposite change, i.e., decrease of membrane fluidity (26,48).

It is well known that adenylate cyclase has a polarized, usually basolateral localization in some epithelial cells (10,15,27,28,38). In the present work we also could demonstrate this phenomenon in fibroblasts. Such heterogeneous membrane polarities are known to occur in several membrane domains both in epithelial cells and in fibroblasts (39,40)

It is an interesting fact that parallel with the radiation-induced increase of enzyme activity this polarized or inhomogeneous distribution becomes modified, i.e., it becomes more uniform. This observation suggests the possibility of a change in the regional distribution of domains among the effects of the radiation-induced membrane alterations. Similar experimental data have been reported in our previous work in connection with radiation-induced modification of the surface distributions of bound concanavalin A and wheat germ agglutinin (40). It appears that besides modification of lectin binding sites, radiation also induces regional changes of some membrane-bound enzymes like adenylate cyclase. The biological significance of this change in distribution is unknown and it also awaits elucidation whether this phenomenon is a direct effect of ionizing radiation on the membrane protein components or a concomitant event of the increase in membrane fluidity (26,48).

We also have preliminary data on the radiation-induced changes in the surface distribution of another enzyme participating in signal transfer through the membranes, guanylate cyclase. According to our observations this enzyme has an apical localization, i.e., opposite to that of adenylcyclase, both in fibroblasts and in human placental trophoblasts (28). This localization also becomes inhomogeneous after irradiation.

Our observations suggesting that cell-to-cell contact areas are predisposed with respect to the cell surface localization of adenylate cyclase are in agreement with the data of Fujimoto et al (12) for the rat myocardium. These authors consider it likely that cAMP has a role in intercellular communication, cellular contacts and in the action of hormones (3,4,25).

We can only speculate about the reasons why enzyme activity increases in these areas after irradiation. It is a well known fact that upon irradiation cell contacts are significantly reduced, and the movement of the cells is increased. This has been reviewed in our previous work (40). Since an increase of the cAMP level is accompanied by a decrease of the mobility of the cells (19) it is conceivable that these changes are aimed at restoring the original state of the culture.
It must be added, however, that according to some views activation of the cAMP system represents a step in the radioprotective mechanism of the organism (36).

As mentioned before, lead precipitates suggesting adenylate cyclase activity have also appeared on the membrane of other cell organelles apart from the plasma membrane. The intracellular localization of this enzyme was described by several authors using different methods (32) and this localization was shown to be present also in fibroblasts.

Acknowledgements

The authors gratefully acnowledge the interest and support of Professor Dr. L.B. Sztanyik, Director General of the Institute.

References

1. Ahn HS, Horowitz SG, Eagle H, Makman MH (1978) Effects on cell density and cell growth alterations on cyclic nucleotide levels in cultured human diploid fibroblasts. Exp. Cell Res. 114, 101-110.

2. Aoyama T, Kimura H, Yamada T (1984) Radiation sensitivity and intracellular cAMP. In "Modification of radiosensitivity in cancer treatment" Academic Press, Japan, Inc., Tokyo, pp. 265-289.
3. Azarnia R, Larsen WJ (1977) Intercellular communications and cancer. In "Intercellular communications", Del Mello WC (ed), Plenum Press. New York, pp. 145-172.
4. Bannai S, Shepard JR (1974) Cyclic AMP, ATP, and cell contact. Nature 250, 62-64.
5. Brooker G, Harper JF, Terasaki WL, Moylan RD (1979) Radioimmunoassay of cyclic AMP and cyclic GMP. Adv. Cyclic Nucleotide Res.Vol. 10, Brooker G, Greengard P, Robinson GA (eds), Raven Press, New York pp. 1-33.
6. Catravas GN, Wright SJ, Trocha PJ, Takenaga J (1981) Radiation effects on cyclic AMP, cyclic GMP, and amino acid levels in the CSF of the primate. Rad. Res. 87, 198-203.
7. Conger AD, Sodicoff M, Samel A (1985) Comparison of cAMP with other radioprotectors against chronic damage to the rat parotid gland. Rad.Res. 102, 99-105.
8. Daniel JW, Oleinick NL (1984a) The participation of elevated levels of cyclic GMP in the recovery from radiation-induced mitotic delay. Int.J.Radiat.Biol. 45, 73-83.
9. Daniel JW, Oleinick NL (1984b) Cyclic nucleotide response and radiation-induced mitotic delay in Physarum polycephalum. Rad.Res. 97, 341-351.
10. Davis WL, Jones RG, Goodman DBP (1987) Electron-microscopic cytochemical localization of adenylate cyclase in amphibian urinary bladder epithelium: Effects of antidiuretic hormone. J. Histochem.Cytochem. 35, 103-111.
11. Friedlander G, LeGrimelec C, Giocondi MC, Amiel C (1987) Benzyl alcohol increases membrane fluidity and modulates cyclic AMP synthesis in intact renal epithelial cells. Biochim.Biophys. Acta 903. 341-348.
12. Fujimoto K, Narimoto K, Ogawa KS, Kondo S, Ogawa K (1985) Review: Cytochemical aspects of gap junction: Enzyme activity, hydrophilic channel and charge. Acta Histochem.Cytochem. 18, 467-481.
13. Hess D, Prasad KN (1981) Modification of radiosensitivity of mammalian cells by cyclic nucleotides. Life. Sci. 29, 1-4.
14. Hunt WA, Dalton TK (1980) Reduction in cyclic nucleotide levels in the brain after a high dose of radiation. Rad.Res. 83, 210-215.
15. Hwang BH, Liu DK (1985) Heterogeneous localization of adenylate and guanylate cyclases in R3230AC rat mammary adenocarcinoma cells. Virchows Arch. (Cell Pathol.) 49, 307-315.
16. ICRU Report 10b (1964) Physical aspects of irradiation. Handbook, 85 (National Bureau of Standards p. 12).
17. ICRU Report 23 (1973) Measurements of adsorbed dose in a phantom irradiated by a single beam of X- or gamma rays. National Bureau of Standards, Washington DC, p. 5.
18. Jacobs KH, Aktories K, Schultz G (1984) Mechanisms and components involved in adenylate cyclase inhibition by hormones. Adv. Cyclic Nucleotide Res. Vol. 17, Greengard P, Robinson GA (eds), Raven Press, New York pp. 135-143.
19. Katzin WE, Gershman H (1984) Control of cell mobility by cyclic AMP. J. Cell Sci., 65, 177-192.
20. Köteles GJ, Kubasova T, Varga L (1976) H-3-Concanavalin-A binding of X-irradiated human fibroblasts. Nature 259, 507.
21. Köteles GJ, Kubasova T, Somosy Z, Horváth M (1983) Derangement of cellular plasma membranes due to non-lethal radiation doses. In "Biological effects of low level radiation". International Atomic Energy Agency, Vienna, pp. 115-129.
22. Kubasova T, Köteles GJ, Somosy Z, Sinkovics I (1986) Use of cell membrane as possible biological indicators of radiation injury. In "Biological indicators of radiation dose assessment". Kaul A, Dehos A, Bögl W, Hinz G, Kossel F, Schwarz ER, Stamm A, Stephan G (eds), MMW Medizin Verlag, München, pp. 199-203.
23. Kuzin AM, Slozhenikina LV, Ushakova TE (1977) The effect of gamma-irradiation on the adenylate cyclase activity of isolated biomembrane of embryonic liver cells. Dokl.Akad.Nauk.SSSR 233, 978-983.
24. Kuzin AM (1980) Different leading molecular mechanism in case of gamma-irradiation with high and low doses. Izv. Acad.Nauk.SSSR, Ser.Biol., 6, 883-890.
25. Larsen WJ (1977) Gap junctions and hormone action. In "Transport of ions and water in epithelia". Wall BJ, Oschman JL, Moreton B, Gupta B. (eds), Academic Press, London, pp. 331-361.
26. Layko W, Bartosz G (1986) Membrane effects of ionizing radiation and hyperthermia. Int.J.Radiat.Biol., 49, 743-770.
27. Lojda Z (1974) Cytochemistry of enterocytes and other cells in the mucous membrane of the small intestine. In "Intestinal absorption, biomembranes". Vol. 4A, Smith DH (ed), Plenum press, London, pp. 43-122.
28. Matsubara S, Tamada T, Saito T (1987) Ultracytochemical localization of adenylate cyclase, guanylate cyclase and cyclic 3'5'-nucleotide phosphodiesterase activity on the trophoblast in human placenta. Direct histochemical evidence. Histochemistry 87, 505-509.
29. Murad F, Arnold WP, Mittal CK, Braughler JK (1979) Properties and regulation of guanylate cyclase and some proposed functions for cyclic AMP. Adv.Cyclic Nucleotide Res. 11, 175-204.
30. Pastan I, Johson GS, Anderson WB (1975) Role of cyclic nucleotides in

growth control. Ann.Rev.Biochem., 44, 491-522.
31. Pausescu E, Poposcu M, Paun C, Teodosiu T (1976) Dynamic of the changes in the tissular levels of cyclic AMP after cobalt 60-gamma-irradiation. Strahlentherapie 151, 165-171.
32. Poeggel G, Luppa H, Berstein HG, Weiss J (1984) Histochemistry of adenylate cyclase. Intern.Rev.Cytol., 89, 35-64.
33. Prasad KN (1972) Radioprotective effect of prostaglandin and an inhibitor of cyclic nucleotide phosphodiesterase on mammalian cells in culture. Int.J.Radiat. Biol., 22, 187-189
34. Purohit SC, Bisby RH, Cundal RB (1980) Structural modification in gamma irradiated human erythrocyte membranes. Int.J. Rad.Biol., 38, 147-166.
35. Rillema JA (1975) Cyclic nucleotides and the effect of prolactin on uridin incorporation int RNA in mammary gland explants of mice. Horm.Metab.Res., 7, 45-49.
36. Sakar SR, Singh LR, Uniyal BP, Chaudhuri BN (1984) Effect of ionizing radiation on tissue cyclic AMP and GMP in experimental rats. Strahlentherapie 160, 631-635.
37. Salesse R, Garnier J (1984) Andenylate cyclase and membrane fluidity. The repressor hypothesis. Molec. Cell Biochem. 60, 17-31.
38. Schwartz IL, Shlatz LJ, Kine-Safran E, Kinne R (1974) Target cell polarity and membrane phosphorylation in relation to the mechanism of action of antidiuteric hormone. Proc.Natl.Acad.Sci., USA 71, 2595-2599.
39. Simons K, Fuller SD (1985) Cell surface polarity in epithelia. Ann.Rev. Cell Biol., 1, 243-288.
40. Somosy Z., Kubasova T, Köteles GJ (1987) The effects of low doses of ionizing radiation upon the micromorphology and functional state of cell surface. Scanning Microsc. 1, 1267-1278.
41. Sołtysiak-Pawluczuk D, Bitny-Szlachto S (1976) Effects of ionizing radiation and cysteamine (MEA) on activity of mouse spleen adenyl cyclase. Int.J.Rad.Biol., 29, 549-553.
42. Sudár F, Csaba G (1986) Simultaneous electron microscopic detection of gold labelled ligand and adenylate cyclase. Influence of Concanavalin-A on adenylate cyclase activity of fibroblasts. Histochemistry 84, 271-274.
43. Sutherland EW, Rall TW (1960) Relation of adenosine-3'5' phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmacol.Rev., 12, 265-283.
44. Tenang EM, McCaldin B (1987) Effects of sera types and cell density on the concentration of cyclic nucleotides in normal and simian virus transformed mouse fibroblasts. Biochem.Intern. 15, 677-685.
45. Traocha PJ, Catravas GN (1980) Variation in cyclic nucleotide levels and lysosomal enzyme activities in the irradiated rat. Rad.Res., 83, 658-667.
46. Traocha PJ, Catravas GN (1983) Effect of radioprotectant WR 2721 on cyclic nucleotides, prostaglandins, and lysosomes. Rad.Res., 94, 239-251.
47. Wagner RC, Bitencky MW (1977) Adenylate cyclase. In "Electron microscopy of enzymes" Vol. 2, Hayat MA (ed), Van Nostrand Reinhold, New York, pp. 110-131.
48. Yonei S, Todo T, Kato K (1979) Evidence for a change in fluidity of erythrocyte membranes following X-irradiation by measurement of pyrene excimer fluorescence. Radiat.Res., 80, 31-45.

Discussion with Reviewers

T.M.Seed: Is the loss of the basolateral distribution of adenyl cyclase following irradiation associated with either the loss of attachment to the substratum or loss/change in cell-to-cell interactions?
Authors: Distribution of membrane domains on cell surface on several occasions are regulated by cell contacts (Herzlinger DA and Ojakian GK, 1984, J. Cell Biol., 98, 1777-1787, Rodriguez - Boulan E et al., 1983, J.Cell Biol., 96, 866-874). Since the irradiation has effects on cell contacts (40), there are also possibilities for such relationship.

J.G.Szekely: Is it possible that the radiation-induced adenylate cyclase and cAMP activity is a cell cycle effect and that the increase is a result of division delay?
Authors: Yes. It may be as Daniel and Oleinick (8,9) proposed such connection.

J.G.Szekely: You mention that in some cases enzyme activity appeared in cell organelles. What was the dose and time dependence of this increased activity?
Authors: The appearance of enzyme activity in cell organelles could be detected both in control and irradiated cells. Special radiation-induced alterations were not observed, i.e., the amount of lead precipitates which were seen in cell organelles did not change after X-irradiation.

J.S.Hanker: I wonder whether the observed changes in adenyl cyclase activity and cAMP levels of these cells could be related to changes in their collagen, mucopolysaccharide or acid mucopolysaccharide synthesis or secretion resulting from ionizing radiation.
Authors: Probably, there are some relationships, since the forskolin or phosphodiesterase inhibitors can stimulate synthesis of sulphated-proteoglycans through intracellular cAMP accumulation (Malemud CJ et al. 1986, J.Cell Physiol., 129, 51-59.