1 Oshima, S., J. Imp. Fish. exp. St. *2* (1931) 139.
2 Iwao, T., Jikken yakub zassi *10* (1936) 357.
3 Schweiger, G., Arch. FischWiss. *8* (1957) 54.
4 Zaba, B.N., and Harris, E.J., Comp. Biochem. Physiol. *61C (1978)* 89.
5 Agrawal, S.J., and Srivastava, A.K., Toxicology *17* (1980) 97.
6 Chandra, S.V., Acta pharmac. tox. *29* (1971) 75.
7 Chandra, S.V., Ara, R., Nagar, N., and Seth, P.K., Acta. biol. med. germ. *30* (1973) 857.
8 Seth, P.K., Nagar, N., Husain, R., and Chandra, S.V., Envir. Physiol. Biochem. *3* (1973) 263.
9 APHA, AWWA and WPCF, Standard Methods for the Examination of Water and Wastewater, 13th edn. Washington, DC 1971.
10 Sangalang, G.B., and O'Halloran, M.J., Biol. Reprod. *9* (1973) 394.
11 Kar, A.B., and Das, R.P., Acta biol. med. germ. *5* (1960) 153.

0014-4754/83/111309-02$1.50+0.20/0

The effect of low doses of X-ray irradiation on cAMP level in Chinese hamster fibroblasts

B.A. Raev, Yu.Yu. Chirkov and I.M. Parkhomenko

Laboratory for Radiation Biophysics, Biological Faculty, Moscow State University, 117234 Moscow (USSR), October 6, 1981

Summary. The effect of a stimulating dose of 0.15 Gy on the cyclic adenosine 3′,5′ monophosphate system has been studied. A rapid change is shown in intracellular level of cAMP and in the response of the system to a β-adrenoagonist.

In recent years biological effects of low-level radiation have been the subject of an increasing number of papers. Despite some contradictory early results, the main idea of a stimulating effect is now widely accepted. The latter has been tested on a wide range of subjects at different biological levels[1-3]. Kalendo et al.[4,5] reported in several successive papers on the stimulation of DNA synthesis and mitotic activity after 0.10 Gy of 137_{Cs} radiation. Manzygin et al.[6] found stimulation of cellular proliferation when a synchronized culture was irradiated in the G_1 phase. The above experiments were carried out on Chinese hamster fibroblasts.

Our own experiments show a stimulation of the initial adhesion to substrate of cultured cells after X-ray irradiation at low doses[7]. A maximum effect of approximately 140% (compared to the control) was obtained with a dose of 0.15 Gy. The same dose was used in our experiments described below.

It is well known that cAMP plays a significant role in the regulation of cellular proliferation[8,9]. It also seems clear that the cAMP level correlates positively with cell-to-substrate adhesiveness at least in cells that have become attached[10]. That is why we have studied the direct effect of a stimulating dose of X-ray irradiation on the cAMP level. The ability of the cAMP system to respond to treatment with β-adrenoagonist was studied simultaneously.

Materials and methods. Chinese hamster fibroblastic cells BAB-II-d-ii-FAF-28, aneuploidal clone 431, were grown in flasks containing 250 ml of growth medium Eagle+199 (1:1) supplied with 20% bovine serum and antibiotics (penicillin 100 units/ml and streptomycin 100 mg/ml). Cells in late log phase were washed twice with Hanks' solution, incubated for several minutes with 0.02% EDTA at 37 °C and collected, also in Hanks' solution, by simple shaking. Samples for cAMP determination contained 10^7 cells; at least 3 samples were used for each point in an

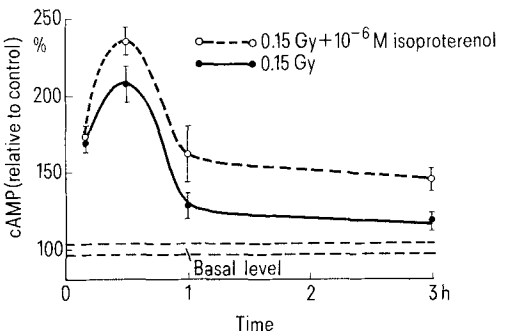

Figure 2. Changes of the intracellular cAMP level after low-dose X-ray irradiation with and without subsequent isoproterenol treatment. The basal cAMP concentration (unirradiated, not isoproterenol treated cells) is 3.35±0.27 pmoles/10^6 cells.

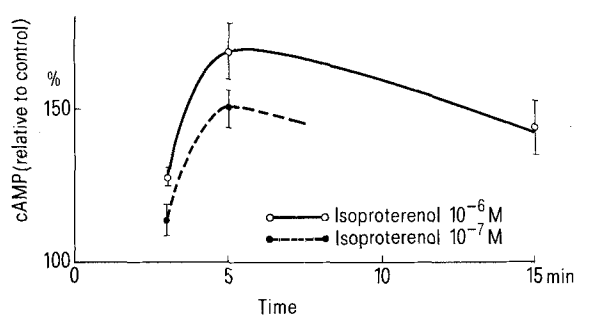

Figure 1. Activation of cAMP production after treatment with isoproterenol. The abscissa shows the incubation time. Concentrations of 10^{-5} M isoproterenol and higher were found to be toxic.

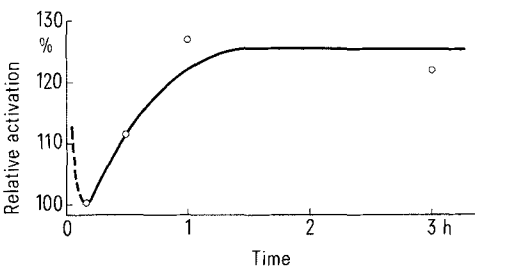

Figure 3. Changes in the relative activation – the ratio between the intracellular cAMP levels in irradiated cells with and without subsequent isoproterenol incubation (0.15 Gy+10^{-6} M isoproterenol): 0.15 Gy. For unirradiated cells the activation of cAMP production with isoproterenol is approximately 170%.

experiment. Cells were irradiated with the aid of a RUM-II Roentgen device (180 kV; 15 mA; 1.0 mm Al + 0.5 mm Cu) at 0.05 Gy/min. Intracellular cAMP level was measured according to the 'Amersham' procedure with the 'Amersham' cAMP assay kit. As a β-adrenoagonist we used isoproterenol (DL-isopropylarterenol) from 'Serva'. A 5-min incubation time at 37 °C prior to cAMP determination was chosen as optimal. Isoproterenol concentration was 10^{-6} M (fig. 1).

Results and discussion. It appears that a stimulating dose of X-ray irradiation (0.15 Gy) shows a drastic effect on the intracellular level of cAMP (fig. 2). In the control cells the level of cAMP was measured to be 3.35 ± 0.27 pmoles/10^6 cells, and we could only assume that the increase starts either immediately after or in the course of irradiation. Because of technical problems our first determination of cAMP level was carried out 10 min after irradiation. At that time the cAMP content was already 160% compared to the control. A maximum 2-fold increase is observed after 30 min followed by a fast decrease, which slows down after 1 h. The intracellular level of cAMP almost returns to its basal level after 3 h.

Irradiation affects not only the cAMP level, but also the reaction of the system to β-adrenoagonist. The dotted-line curve in figure 2 represents the changes of cAMP level in irradiated cells treated with isoproterenol. Figure 3 shows the relative activation i.e. the ratio between cAMP levels in irradiated cells with and without isoproterenol incubation (0.15 Gy + 10^{-6} M isoproterenol: 0.15 Gy). It is well known that the biological activity of the isoproterenol is accomplished only through the membrane β-adrenoreceptors with further activation of the adenylate cyclase. Thus figures 2 and 3 reveal a rapid and strong influence of low-level X-ray irradiation on the β-adrenergic activation of the adenylate cyclase.

An attempt to co-ordinate our results with available data shows a positive correlation between the increase in intracellular level of cAMP and the reinforcement of cell-to-substrate adhesion, although data on initial adhesion are somewhat contradictory. Difficulties arise when considering stimulation of cellular proliferation. A lot of papers show that almost always when cells are stimulated to proliferate, the effect is inhibited by cAMP analogues or agents which increase the cAMP level[9,11]. If cells undergo transition from quiescence to proliferation the intracellular level of cAMP decreases immediately[8,12]. Only in the late G_1 phase could an increase in the cAMP level have a positive or stimulating role, possibly as a step in the normal program of the cell cycle[13]. That is also the case described by Manzygin et al.[6]. Friedman[13] also quotes reports of cAMP stimulating mitotic activity for HeLa cells, but this effect is not verified for other cell types.

Earlier, applying fluorescent probes, we observed[14] conformational changes in the cellular outer membrane after low doses of X-ray irradiation. They have the same time-course as the cAMP effects described and it is quite possible that these changes are responsible for the disturbance of the process of β-adrenergic activation of the adenylate cyclase, as well as for its stimulation to catalize the production of intracellular cAMP. The possibility of activation of membrane-bound enzymes by conformational changes in the membrane structure has been discussed elsewhere[15].

1 Arnauld, Y., 3rd Int. Congr. Radiat. Res., Cortina d'Ampezzo 1966; abstract p. 93.
2 Planel, G., Soleilhavoup, J.P., Tixador, R., Croute, F., and Richoilley, G., in: Biology and Environment. Effects of Low-level Radiation, vol. I, p. 127. IAEA, Vienna 1976.
3 Kuzin, A.M., Vagabova, M.E., and Primak-Mirolyubov, V.N., Radiobiologia *17* (1979) 37.
4 Kalendo, G.S., Yarmonenko, S.P., and Vinskaja, V.P., Radiobiologia *11* (1971) 871.
5 Kalendo, G.S., and Zhurbitskaja, V.A., Tsitologia *16* (1974) 1365.
6 Manzygin, Yu.A., Nasarova, L.F., and Kuzin, A.M., Radiobiologia *21* (1981) 109.
7 Raev, B.A., and Parkhomenko, I.M., Sb. Mat. IVth Conf. Bolg. Asp. in USSR, vol. 11, p. 637. Moscow 1981.
8 Otten, J., Johnson, G.S., and Pastan, I., J. biol. Chem. *247* (1972) 7082.
9 Bombik, B.M., and Burger, M.M., Exp. Cell Res. *80* (1973) 88.
10 Willingham, M.C., Int. Rev. Cytol. *44* (1976) 319.
11 Frank, W., Exp. Cell Res. *71* (1972) 238.
12 Oey, J., Vogel, A., and Pollack, R., Proc. natl Acad. Sci. USA *71* (1974) 694.
13 Friedman, D., Physiol. Rev. *56* (1976) 4.
14 Raev, B.A., and Parkhomenko, I.M., in: Dokl. MOIP, p. 47. Ed. O.R. Kolis. Moscov 1981.
15 Ljachnovitch, G.V., Kaler, G.V., and Konev, S.V., Veszi AN BSSR, ser. bial. navuk *6* (1981) 80.

0014-4754/83/111310-02$1.50 + 0.20/0

Mast-cell heterogeneity in the rat

J. Damas and J. Lecomte

Institut Léon Fredericq, Physiologie humaine, normale et pathologique, University of Liège, B-4020 Liège (Belgium), January 24, 1983

Summary. In the rat, O-hydroxy ethyl rutoside derivatives release histamine and serotonin from skin mast-cells, but not from peritoneal mast-cells. These cellular populations do not exhibit identical pharmacological properties.

The i.v. injection of O-hydroxy ethyl rutoside derivatives[1] (OHRD) in rats of various strains induces a fall in systemic arterial blood pressure and a generalized cutaneous oedema[2-4]. Plasma levels of histamine and 5-hydroxytryptamine are simultaneously increased. OHRD-induced hypotension and oedema are inhibited by promethazine and methysergide. Thus 'in vivo' OHRD-induced reactions are related to a release of mast-cell amines. OHRD act in the same way as dextran or ovomucoid.

We report here on a comparison of the responses, to these amine liberators, of rat peritoneal and cutaneous mast-cells.

Materials and methods. Peritoneal mast-cells. Rats were anesthetized by s.c. injection of sodium pentobarbital