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 $\beta$-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 $3.35 \pm 0.27$ 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 $\beta$-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 $\beta$-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 $\beta$-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 $\beta$-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

(Nembutal® 30 mg/kg). 5 ml of a Tyrode solution with heparin (10 µg/ml) was injected i.p. The abdomen was gently massaged for 1 min and the fluid withdrawn through a midline incision. Fluid samples from 5 to 10 rats were pooled and the crude cell suspension was exposed to OHRD for 30 min at 37 °C. The cell-suspension was then centrifuged at 1000 × g for 5 min and the amine level in the supernatant assayed.

Cutaneous mast-cells. Two methods were used for testing the reactivity of cutaneous mast-cells. First, the rat hind quarter preparation was perfused according to Feldberg and Mongar preparation[5]. Second, skin flaps were removed from the hind paws. The flaps were chopped with scissors, washed with cold Tyrode solution, blotted with filter paper, weighed and homogenized. The homogenate was centrifuged at 250 × g for 5 min to remove skin fragments. The suspension, which contained some intact mast-cells, was then diluted with Tyrode solution to 50 mg/ml. After 30 min of incubation at 37 °C with or without OHRD, the suspension was centrifuged at 1000 × g for 5 min. The supernatant was then assayed for its amine levels.

Amines assay. Histamine and 5-hydroxytryptamine concentration were measured by bioassay using 2 sets of 3 pieces of tissues in each case (guinea-pig ileum and rat stomach strip[6]) superfused with Tyrode solution in the presence or in the absence of cyproheptadine and mepyramine ($5 \cdot 10^{-7}$).

*Results and discussion.* Injection of 1–10 mg of OHRD in the aortic cannula of a perfused hind quarter preparation provokes the appearance of free histamine in the effluent of the vena cava (0.2–20 µg in 10–15 min). Skin histamine stores are depleted.

'In vitro' addition of OHRD (50 µg–1 mg · ml$^{-1}$) for 30 min to rat peritoneal mast-cells in Tyrode's solution (glucose 5.6 mM, $CaCl_2$ 1.8 mM), induces neither degranulation as observed using phase-contrast microscopy, nor amine release as detected by superfusion. OHRD with rat plasma (5–25% vol.) or OHRD plus $CaCl_2$ 3 mM are also inactive.

Similarly, 'in vitro' heterogenous cell suspensions containing some intact mast-cells, prepared from skin-flaps from the hind-paws of Wistar rats, release large amounts of histamine and serotonin when exposed to 300 µg to 1 mg · ml$^{-1}$ of OHRD (table).

Compound 48/80 (1–10 µg · 100 g$^{-1}$ or 1–10 µg · ml$^{-1}$), used to test the viability and the amine-releasing properties of the preparations, is equally active on peritoneal mast-cells, isolated skin mast-cells and perfused hind quarters.

Therefore, skin mast-cells, 'in vivo' as well as 'in vitro' release histamine and serotonin in the presence of OHRD in concentrations which are completely inactive on peritoneal mast-cells 'in vitro'.

In the same species, qualitative pharmacological differences appear to exist between peritoneal mast-cells and connective skin mast-cells. So it would not be correct to draw conclusions about the properties of one population on the basis of the responses of the other. This precludes any generalization and extrapolation, especially for clinical purposes. This conclusion is in agreement with Barrett and Pearce's results[7] comparing rat peritoneal and pleural mast-cells.

Histamine and serotonin (ng · ml$^{-1}$) detected in the supernatant of rat skin mast-cells or of rat peritoneal mast-cells (mean ± SEM)

| | | Histamine | Serotonin | n |
|---|---|---|---|---|
| Skin | | | | |
| Control | | 1230 ± 118 | 12.5 ± 2.7 | 6 |
| 48/80 | 10 µg ml$^{-1}$ | 3200 ± 84* | 71.2 ± 10.8* | 4 |
| OHRD | 1 mg ml$^{-1}$ | 3415 ± 140* | 67 ± 10.5* | 6 |
| Peritoneum | | | | |
| Control | | 110 ± 14 | 5 ± 2 | 6 |
| 48/80 | 10 µg ml$^{-1}$ | 1325 ± 137* | 86 ± 5* | 6 |
| OHRD | 100 µg ml$^{-1}$ | 100 ± 9 | 5 ± 2 | 6 |
| | 300 µg ml$^{-1}$ | 110 ± 12 | 5 ± 2 | 6 |
| | 1 mg ml$^{-1}$ | 105 ± 14 | 5 ± 2 | 6 |

n, Number of assays; * significantly different from the control, $p < 0.01$; Student's t-test for paired values.

1 Courbat, P., Uhlmann, G., and Guerne, R., Helv. cli. Acta *49* (1966) 1203.
2 Lecomte, J., Cession-Fossion, A., Baeckeland, E., and Van Cauwenberge, H., Bull. Acad. R. Méd. Belg. *11* (1971) 271.
3 Lecomte, J., and Van Cauwenberge, H., C.r. Soc. Biol. *167* (1973) 396.
4 Damas, J., and Lecomte, J., C.r. Soc. Biol. *174* (1980) 216.
5 Feldberg, W., and Mongar, J., Br. J. Pharmac. *9* (1954) 197.
6 Vane, J., Br. J. Pharmac. *35* (1969) 209.
7 Barrett, K., and Pearce, F., Agents Actions *12* (1982) 186.

0014-4754/83/111311-02$1.50+0.20/0


## The chromosome complements of two species of *Gobius* (Teleostei, Perciformes)

G. Thode and M.C. Alvarez[1]

*Departamento de Genética, Facultad de Ciencias, Universidad de Málaga, Malaga (Spain), February 25, 1983*

*Summary.* The karyotypes of *G. bucchichi*, 2n = 44, and *G. cruentatus*, 2n = 46, are described. No chromosome differences have been revealed between males and females of either of these gonochoristic species. The results are compared with data from other species of the same genus.

The large number of species (over 600) described for the genus *Gobius*, the fact that Gobiidae show very similar morphological patterns, and their high intraspecific variability, can make identification of these species on the basis of only morphological parameters very difficult. In spite of this phenotypic similarity, however, karyological studies in this genus show that the karyotype can be used to identify each species unequivocally. In the present work, the karyotypes of *G. bucchichi* and *G. cruentatus* are described for the 1st time, as a part of a broad study carried out in our laboratory on the cytogenetics of this group.

*Material and methods.* Two males and 6 females of *G. bucchichi* (Steindachner, 1870) and 1 male and 3 females of *G. cruentatus* (Gmelin, 1789) were collected from the southern Mediterranean coast of Spain. The Giemsa-air dried metaphase plates were made from spleen, kidney and gonads, following the procedure described by Alvarez et al.[2] with slight modifications.