Role of calcium in the histamine release induced by D-galactosamine from rat mast cells[1]

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Summary. Rat peritoneal mast cells were isolated and purified by differential centrifugation in Ficoll. Cells pooled from three to four rats were suspended at approximately 10^6 cells/ml in a buffered salt solution and incubated for 1 h at 37 °C in 300 µl volumes in the absence or presence (9×10^{-4} M) of calcium chloride. Addition of D-galactosamine hydrochloride (DGM; 2.8×10^{-4} M) caused (in addition to basal release) a mean ± SEM percent histamine release of 15.7 ± 5.2 in the presence of Ca^{++} and 19 ± 4.9 in the absence of Ca^{++} ($p > 0.05$). It is suggested that D-galactosamine does not require extracellular Ca^{++} for the release of histamine from the rat mast cell.

Key words. Mast cells; histamine; D-galactosamine; extracellular Ca^{++}.

Histamine may be selectively released from the mast cells by a wide variety of pharmacological and immunological stimuli[3]. Some of these act by increasing the permeability of the cell membrane to extracellular calcium ions[4]; others (like compound 48/80) release histamine by mobilizing intracellular calcium stores[5,6].

We demonstrated earlier that D-galactosamine hydrochloride (DGM) has the ability to release histamine from the paw skin of the rat[7,8]. It is, however, not known whether the action of DGM was dependent on the presence of extracellular calcium. The present in vitro study was therefore undertaken to show whether extracellular Ca^{++} is necessary for DGM-induced histamine release from rat peritoneal mast cells.

Materials and methods. Rat peritoneal mast cells were isolated and purified by differential centrifugation in Ficoll[9]. Cells pooled from three to four rats were suspended at approximately 10^6 cells per ml buffered salt solution[10] but with 0.9 mM $CaCl_2$. They were incubated in 300 µl volumes at 37 °C for 1 h and histamine release was estimated in the supernatant. In order to study the effect of calcium on histamine release, a similar number of incubations was carried out in calcium-free media. The water used in these studies was double distilled and the chemicals were of analytical quality. DGM was added just prior to incubation to give a final concentration of 0.56×10^{-4} to 7×10^{-3} M. At the end of the incubation, the histamine content of the supernatants and of representative samples of cell suspensions were estimated using a fluorimetric method[11].

Results. The table shows that cells incubated without the addition of DGM released mean of 9.43% histamine in the presence of Ca^{++} in the incubating medium.

The addition of DGM at a concentration of 0.56×10^{-4} moles/l caused 17.46% release of histamine in the presence and 12.10% release in the absence of Ca^{++} but the two values were not significantly different ($p > 0.05$) from each other. The increase in DGM concentration to 2.8×10^{-4} moles/l further enhanced the release of histamine from mast cells but the values in the presence of Ca^{++} were similar to those obtained in the absence of Ca^{++}. A further fivefold increase in DGM concentration (1.4×10^{-3} moles/l) in the medium, however, failed to enhance the release of histamine either in the presence or in the absence of Ca^{++} from the incubating medium. At a still higher concentration (7.0×10^{-3} moles/l) DGM failed to release any histamine from mast cells. Over the whole range of DGM concentration, the percent histamine releases in the two groups were very similar to one another. Furthermore, the release of histamine induced by 1.4×10^{-3} moles/l DGM was not significantly different from that induced by 2.8×10^{-4} moles/l DGM in the medium ($p > 0.05$ for both comparisons).

Discussion. Pearce and his colleagues[12] have shown that at least three pools of calcium are important in histamine secretion from the mast cells. Firstly, calcium loosely bound to the outer cell membrane may normally be utilized during the anaphylactic and other reactions. This calcium may migrate into the cell cystosol through appropriate channels and so initiate exocytosis. Secondly, calcium firmly bound to within the cell membrane is activated by the anaphylactic agents and other stimuli[13]. Finally, calcium bound to regulatory sites in the membrane may modulate the release process.

D-galactosamine induced histamine release from isolated rat mast cells. The absence of Ca^{++} from the extracellular medium did not produce any significant effect on the release of histamine. This is unlike the antigen-induced release of histamine which requires the presence of extracellular Ca^{++} for secretion of histamine from mast cells[14].

D-galactosamine is an amino sugar and in electrophoresis studies behaves as a basic compound[15]. The mechanism of D-galactosamine-induced histamine release from mast cells is not known. The other basic compounds known to be histamine releasers act at least by two different mechanisms. One requires the presence of Ca^{++} and is susceptible to anoxia and also to pH changes in the medium[3]. The other mechanism involves a detergent-like action which produces disruption of perigranular and plasma membranes as well as alterations in the mitochondria and the reticulum of the mast cell[16]. While it is possible that DGM acts by the later mechanism, the possibility that it utilizes

D-galactosamine (DGM)-induced release of histamine from mast cells incubated in the presence and absence of Ca^{++} from the incubating medium (means ± SEM; n = 8 in each group)

DGM (moles/l)	In the presence of Ca^{++}			In the absence of Ca^{++}		
	Total histamine (µg/10^6 cells)	Histamine released in the supernatant (µg/ml)	Age release (%)*	Total histamine (µg/10^6 cells)	Histamine released in the supernatant (µg/ml)	Age release (%)*
—	7.36 ± 0.726	—	—	8.82 ± 0.656	—	—
—	—	0.68 ± 0.099	9.43 ± 1.05	—	0.78 ± 0.122	8.84 ± 1.379
		Basal release	Basal		Basal release	Basal
0.56×10^{-4}	—	1.24 ± 1.149	17.46*** ± 1.965	—	1.08 ± 0.242	12.10 ± 2.314
2.8×10^{-4}	—	1.62 ± 0.153**	25.14*** ± 5.160	—	2.50 ± 0.495**	28.09*** ± 4.896
1.4×10^{-3}	—	1.55 ± 0.218**	23.94*** ± 5.713	—	2.09 ± 0.347**	22.81*** ± 2.471
7.0×10^{-3}	—	0.36 ± 0.98	5.10 ± 1.320	—	0.52 ± 0.094	6.31 ± 1.397

* Calculated from individual experiments. ** $p < 0.05$, values compared with the basal release values in the group. *** $p < 0.05$, compared with the percent basal release in the group.

endogenous Ca^{++} to trigger histamine secretion from the mast cells cannot, however, be excluded. In this regard, it is noteworthy that some of the basic compounds like compound 48/80 and peptide 401 (the mast cell degranulating peptide from bee venom) give virtually optimal release in the absence of extracellular calcium[6].

The mechanism of D-galactosamine-induced histamine release from isolated rat peritoneal mast cells may be related to the mobilization of intracellular Ca^{++} from one or more of the calcium pools described by Pearce and his colleagues[12]. Further experiments using other divalent cations (e.g. Co^{++}, Mn^{++}) and organic calcium blockers to block calcium influx are suggested. These would help us to elucidate further the mechanism of action of D-galactosamine. D-galactosamine used in higher concentrations (7×10^{-3} M) failed to cause histamine release from the mast cells; this could be due to an induction of supra-optimal concentrations of Ca^{++} from the intracellular calcium pools which, in turn, inhibited histamine release. D-galactosamine is a derivative of 2-deoxy-D-galactose, which is a weak inhibitor of histamine release from mast cells[10]. It is therefore not surprising that at a higher concentration (7×10^{-3} M) DGM failed to release histamine from these cells. At this level, it could have acted as a metabolic poison in a way similar to that described for 2-deoxy-D-glucose[17,18].

1 A preliminary analysis of these results was presented at the International Symposium on calcium entry blockers and tissue protection, Rome, 15–16 March 1984.
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Preferential destruction of chloroplast nucleoids in zygotes in green algae *Dictyosphaeria cavernosa* and *Acetabularia calyculus*

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Summary. The preferential destruction of chloroplast nucleoids in young zygotes in the coenocytic alga *Dictyosphaeria cavernosa* and the giant unicellular alga *Acetabularia calyculus* was studied by high resolution epifluorescent microscopy. The chloroplast nucleoids (DNA) in the chloroplast from one of the parents were preferentially destroyed soon after the mating of male and female gametes.
Key words. Preferential destruction; chloroplast nucleoids; *Dictyosphaeria cavernosa, Acetabularia calyculus.*

The unicellular isogamous green alga *Chlamydomonas reinhardtii* shows maternal transmittance of chloroplast genes like higher plants[1]. Therefore, the question whether or not uniparental transmittance of chloroplast genes or chloroplast DNA occurs in giant algae, which seem to be phylogenetically higher than *C. reinhardtii*, needs to be examined.

Previous studies on maternal inheritance in *C. reinhardtii* have shown by means of 4′,6-diamidino-2-phenylindole (DAPI) staining that the chloroplast nucleoids of male origin are destroyed shortly after mating, while the chloroplast nucleoids of female origin remain. Thus preferential destruction may account for the maternal inheritance of chloroplast genes[2]. DAPI staining is a simple and easy method for observing very small amounts of DNA in organelles[3–5].

The present experiments were done to observe the behavior of chloroplast nucleoids in young zygotes in green algae by means of DAPI staining.

Materials and methods. Dictyosphaeria cavernosa (Forssk) Børg was collected from the intertidal zone at Amami Island, Japan, placed in a separate glass vessel containing 100–150 ml of sterilized seawater and incubated according to a method described previously[6]. Swarmers of *D. cavernosa*, released about 1 month after initiation of the culture, were collected by utilizing their positive phototactic response. The sex of the gametes was determined by crossing tests. *Acetabularia calyculus* was originally collected from Wakasa Bay, Japan, and cultured autotrophically by methods described previously[7]. Cysts of *A. calyculus* were isolated from the caps and each cyst was placed in a separate watch glass. The gametes were released in 1–2 days after initiation of the culture. Female and male gametes of *D. cavernosa* and *A. calyculus* were mixed in a watch glass. The newly formed zygotes were fixed in 1% glutaraldehyde dissolved in buffer S[2] at 30, 60, 90 and 120 min after mixing of female and male gametes, and stored at 4 °C. They were stained with DNA fluorochrome DAPI and examined by Olympus BHS-RFK epifluorescent microscopy[2].

Results and discussion. Each gamete of *D. cavernosa* has two flagella about 15 μm long and contains a cell nucleus of 3 μm