Biochemical Pharmacology, Vol. 34, No. 23, pp. 4169–4170, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

SHORT COMMUNICATIONS

Metabolic energy dependent exocytosis of mouse mast cells by lectin of *Dioclea grandiflora* (Mart.)

(*Received* 25 *October* 1983; *accepted* 27 *June* 1985)

A large number of agents has been shown to induce exocytosis of granules from mast cells. Among them, lectins have recently moved to the forefront of biological research. Several lectins have the capacity to trigger mast cells for exocytosis [1–7] although other ones such as Jack fruit lectin did not exhibit such ability [8].

In the present work we used the lectin of *Dioclea grandiflora* (Mart.) [9] to induce exocytosis of a mouse mast cell system. We demonstrated that the activation of mouse mast cells by the lectin of *Dioclea grandiflora* (Mart.) requires metabolic energy as do anaphylactic processes [10–15]. The energy supply was studied by using metabolites and metabolic inhibitors and the reversion of the exocytosis inhibition, besides being tested through a distinct metabolic route, as it has been done up to now, was also tested through the same blocked route.

Methods

All the experiments were carried out by using Swiss mice, kept as inbred lines at the Department of Biochemistry and Molecular Biology of the Federal University of Ceara, Brazil.

All chemicals were of analytical grade.

A lectin from seeds of *Dioclea grandiflora* (Mart.), P_{III}, was prepared according to R. Moreira *et al.* [9].

A pool of peritoneal cells from several mice was collected after intraperitoneal injection of 2 ml Ringer Barron medium [16] and the washed cells were kept in a water bath at 37° for 10 min for temperature and metabolism adjustments [16]. Inhibitors were added after the 10 min of thermo-equilibrium simultaneously with the metabolites. The incubation times are indicated in the corresponding tables and figures. As the time course requirement for maximal activity of lectins is considered to be a physicochemical characteristic [4], a kinetics for mast cell degranulation with *Dioclea grandiflora* lectin was carried out previously: after 6 min the lectin-induced degranulation was already in the plateau. Consequently, after the addition of the lectin, incubation was continued at 37° for a further 6 min. At the end of the reaction, the cells were stained with toluidine blue [16]. Results are expressed as the percentage of mast cells which became degranulated after exposure to the lectin. Controls for spontaneous degranulation without and with inhibitors and metabolites were always performed. The values (5–10%) were deducted from the shown results. The data expressing inhibition of mast cell degranulation were calculated considering as 100% the degranulation induced by 50 μg/ml of the lectin of *Dioclea grandiflora* (Mart.). At least 100 mast cells were counted for each experimental data. The viability of the mast cells in the different experimental conditions was controlled by the trypan blue test.

Results and discussion

Several metabolic inhibitors were used as tools for characterizing the origin of the metabolic energy supply for the lectin induced mast cell exocytosis. The inhibitor of the glycolytic pathway, FNa, by its own had no effect on the exocytosis process. 2–4 DNP, an uncoupler of oxidative phosphorylation and oligomycin, an inhibitor of ATP formation and of electron transport coupled to phosphorylation produced inhibition of the exocytosis. Inhibitors of the electron transport chain at the level of the three sites of ATP formation such as rotenone (site I), antimycin (site II) and KCN (site III) also produced inhibition of mast cell exocytosis. For the above-mentioned inhibitors the I 50% is shown in Table 1.

Table 1. Effect of metabolic inhibitors on *Dioclea grandiflora* lectin induced degranulation of mouse mast cells

Inhibitor	50% Inhibition (I 50%)* (μM)
2.4 DNP	11.00
Oligomycin	0.12
Rotenone	0.11
Antimycin A	0.02
KCN	16.00

After 10 min preincubation, various concentrations of inhibitors were added and incubated for 5 min prior to the addition of 50 μg/ml of the lectin incubated for a further 6 min. Spontaneous degranulations with and without the inhibitors were deducted from the corresponding data.

* I 50% expresses the concentration of each inhibitor inducing a 50% inhibition of mast cell degranulation.

The reversion of the inhibition of the two metabolic routes was carried out by using specific metabolites taking part in each route. In the present experiments, as in other studies for reversion of the uncoupling effect of 2.4 DNP on IgE-mediated mouse and other animal mast cell secretion [12, 13], glucose was shown to be efficient. In such a situation, a reversion of an energy dependent biological process induced by a different metabolic pathway, glycolysis, occurred since the oxidative phosphorylation coupled to the respiratory chain was blocked (Fig. 1). The 2.4 DNP induced inhibition of exocytosis was progressively reversed when glucose concentration was increased up to 5 mM, a concentration lower than those which specifically inhibit the activity of *Dioclea grandiflora* lectin on mast cells (Fig. 1B).

For the first time, to our knowledge, a reversion of the energy dependent mast cell activation was obtained through a reoperation of the electron transport chain previously blocked. That was the case of the mast cell activation induced by lectin of *Dioclea grandiflora*, inhibited by rotenone and, reversed by succinate (Table 2). This metabolite is known to furnish reducing power to the electron transport chain after the blockage point of the rotenone. Thus, the electron transport can flow again with the generation of two molecules of ATP instead of three, as it occurs when the respiratory chain is fully functioning from NADH dehydrogenase to the oxygen. This gain of two molecules

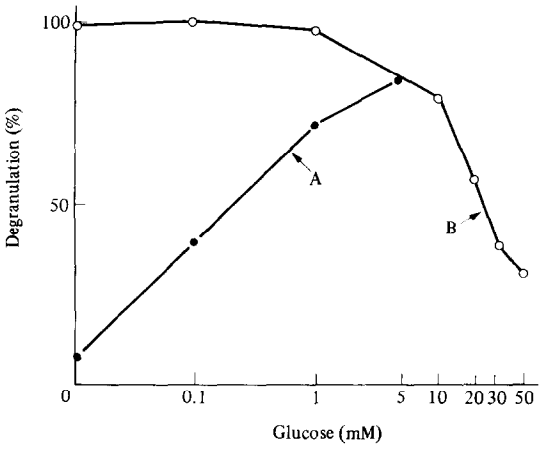

Fig. 1. Effect of glucose in presence and in absence of 2.4 DNP on *Dioclea grandiflora* lectin induced degranulation of mouse mast cells. After the 10 min preincubation, the various concentrations of glucose with (A) and without (B) 10^{-4} M 2.4 DNP were added and incubated for 10 min prior to the addition of the lectin. The other experimental conditions are the same as those indicated for Table 1.

Table 2. Inhibitory effect of rotenone on mast cell degranulation induced by lectin of *Dioclea grandiflora* (Mart.) with reversion by succinate

Rotenone* (μM)	Succinate* (mM)	Degranulation percent inhibition†
—	—	0
0.5	—	83 ± 4.6 (5)
0.5	5	33 ± 3.1 (7)
0	5	8

Same experimental conditions as indicated for Table 1.
* Preincubation with mast cells for 5 min.
† Mean ± S.D., in parentheses number of experiments.

of ATP was efficient enough to reverse the inhibition of the mast cell lectin induced degranulation by rotenone.

Experiments using antimycin A as an inhibitor of the electron flux of the respiratory chain at the level of cytochrome *b* revealed that ascorbate, which is potentially able to furnish reducing power to the respiratory chain after the blocking point of antimycin A, was not able to reverse the exocytosis inhibition caused by the antimycin A. It is possible to assume that the gain of only one molecule of ATP from the Site III does not represent an energy quantity sufficient to reverse the inhibition caused by antimycin A.

A correlation between the ATP level and the mast cell secretory process has been already pointed out [17–20]. Such quantitative requirement for ATP has been emphasized with the results here reported.

The present work, using a new model of inducing mast cell exocytosis by the lectin of *Dioclea grandiflora*, contributes with different aspects to clarify the participation of the energy metabolism in the mentioned process. The operation of fragments of the coupled respiratory chain puts in evidence the question of the amount of energy, a minimum of two molecules of ATP, needed for the mast cell exocytosis.

Acknowledgements—This work was supported by Cooperation Project between C.N.R.S. (France) and C.N.Pq. (Brazil).

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