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Histamine release induced by glucose (mannose)-specific lectins isolated from Brazilian beans. Comparison with concanavalin A

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Abstract. The histamine releasing properties of glucose (mannose)-specific lectins isolated from Brazilian beans was examined. The *Canavalia brasiliensis*, *Dioclea rostrata*, and *Dioclea virgata* lectins induced histamine release in rat peritoneal mast cells similar to concanavalin A. Less potency and efficacy was observed for *Canavalia maritima*, *Dioclea guianensis*, and *Dioclea violacea* while very low activities were seen for the lectins from *Dioclea grandiflora*, *Canavalia bonariensis*, and *Cratylia floribunda*.

The histamine releasing effect was quenched by higher doses of *D. virgata* lectin similar to what was reported for concanavalin A. This effect was abrogated by increasing the concentration of calcium in the incubating medium. As these above proteins have sites that bind calcium, higher doses of the lectins might withdraw the calcium which is essential for the mast cell secretion.

Key words: Histamine release – Lectins – Mast cells

Introduction

Lectins are proteins that bind to carbohydrates and sugar-containing substances in a reversible way [1]. This characteristic enables lectins to produce assorted biological effects like erythrocyte and malignant cell agglutination, induction of mitosis in lymphatic cells among others that emphasizes the importance of these substances [2, 3]. In 1973, Keller [4] reported that concanavalin A, a lectin derived from jack beans, could produce histamine release from rat mast cells harvested from animals previously infected with the worm *Nippostrongyllus brasiliensis*. The absence of effect in cells coming from parasite-free animals, temperature dependence, and inhibition induced by high concentration of carbohydrates indicated a mechanism similar to antigen–antibody reaction, possibly due to a cross-link of Fc regions of the immunoglobulin molecule. Magro and Bennich [5] confirmed this mechanism, although there is evidence that concanavalin A can also induce histamine release in mast cells that have their antibodies removed from cell surface [6]. The histamine release induced in rat mast cells by concanavalin A can be potentiated by phosphatidylserine [6, 7], and is avoided by the absence of external calcium [6, 8]. Concanavalin A stimulates histamine release in mast cells or basophils from various animal species like rabbit, guinea pig, mouse, hamster as well as in human [9]. This fact makes it an important model to study the allergic histamine release allowing non-invasive experiments in humans.

In the present paper, we studied in rat peritoneal mast cells (RPMC) the histamine releasing effects of the lectins isolated from Brazilian beans belonging to the same tribe and sub-tribe (*Phaseoleae* and *Diocleineae*), and known to be specifically inhibited by glucose, mannose, and derivatives [10–14]. The histamine-releasing properties of these lectins were compared to concanavalin A.

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Methods

Animals and sensitization

RPMC from Wistar rats (180–300 g) and a Tyrode solution (pH adjusted to 7.4) containing the following composition (m*M*): NaCl (137), KCl (2.7), $CaCl_2$ (1), NaH_2O_4 (0.4), $NaHCO_3$ (12) and glucose (5.5) were used throughout these experiments. The animals were sensitized to ovalbumin by subcutaneous injection of 1 ml of a solution containing 1 mg of ovalbumin plus 200 mg of aluminium hydroxide. The animals were used 21–51 days after the sensitization.

Cell suspensions and incubations

The cellular suspensions containing mast cells were prepared by direct lavage of the peritoneal cavity with a Tyrode solution containing heparin (5 units/ml), centrifugation ($150 \times g$, 5 min, room temperature), and twice lavage in heparin-free solution. For the histamine release experiments, the cells (0.5 ml) were allowed to warm up for 5 min in a water bath (37 °C), the different concentrations of lectins were added (20 µl), and the samples were incubated for 10 min before stopping the reaction by the addition of cold Tyrode (4 °C, 1.5 ml). The cells were centrifuged ($150 \times g$, 4 °C,

5 min), the supernatants were poured in clean tubes, and equal volumes of Tyrode were added to the cells pellets. All the samples received perchloric acid in a final concentration of 0.4 *M*.

Lectin purifications

The nine lectins studied were isolated from *Canavalia brasiliensis*, *Dioclea rostrata*, *Dioclea virgata*, *Dioclea violacea*, *Canavalia maritima*, *Dioclea guianensis*, *Canavalia bonariensis*, *Dioclea grandiflora*, and *Cratylia floribunda*. Except concanavalin A that was purchased from Sigma, all the lectins were isolated by affinity chromatography on the same type of matrix essentially as reported before [10–13]. Briefly, the whole mature seeds were ground and the protein extracts were prepared by treating the seed meals with 0.15 *M* NaCl (1:10, w/v). The insoluble material was pelleted by centrifugation at $10000 \times g$ at 5 °C for 20 min. The lectin-rich fractions obtained by precipitation of the extracts with ammonium sulphate were applied to a Sephadex G-50 column equilibrated with 0.15 *M* NaCl containing 5 m*M* $CaCl_2$ plus 4 m*M* $MnCl_2$. After removing unbound material, the lectins were desorbed from the column with 0.1 *M* glucose added to the equilibrium solution or with 0.05 *M* glycine-HCl buffer, pH 2.6. To avoid any residual sugar bound to the lectin desorbed with glucose, dialysis against 0.5 *M* acetic acid (1 h) was carried out before exhaustive dialysis with distilled water. The lectins obtained by this procedure are pure compounds. The degree of purification of each particular lectin was checked by PAGE-SDS (sodium dodecyl sulphate ammonium polyacrylamide gel electrophoresis). All of them are concanavalin A-like lectins composed of a main protein band (alpha subunit) and two fragments (beta and gamma) [15]. The lectins were dissolved in saline (NaCl 0.9%) just before the experiments.

Histamine assay

The histamine content in the pellets and supernatants were extracted and assayed fluorometrically using an automated apparatus (Technicon Autoanalyser II). The lectins we used did not interfere with the fluorescence produced during the assay of histamine.

Results and statistical analysis

The results were expressed as mean ± standard errors of the percentage released (supernatant) from the total histamine (supernatant plus pellets) existing in the cells, discounting the spontaneously released value (less than 15%). Where specified, the data were analyzed by the Student's *t*-test for paired samples. The differences were considered significant at the level of $P < 0.05$.

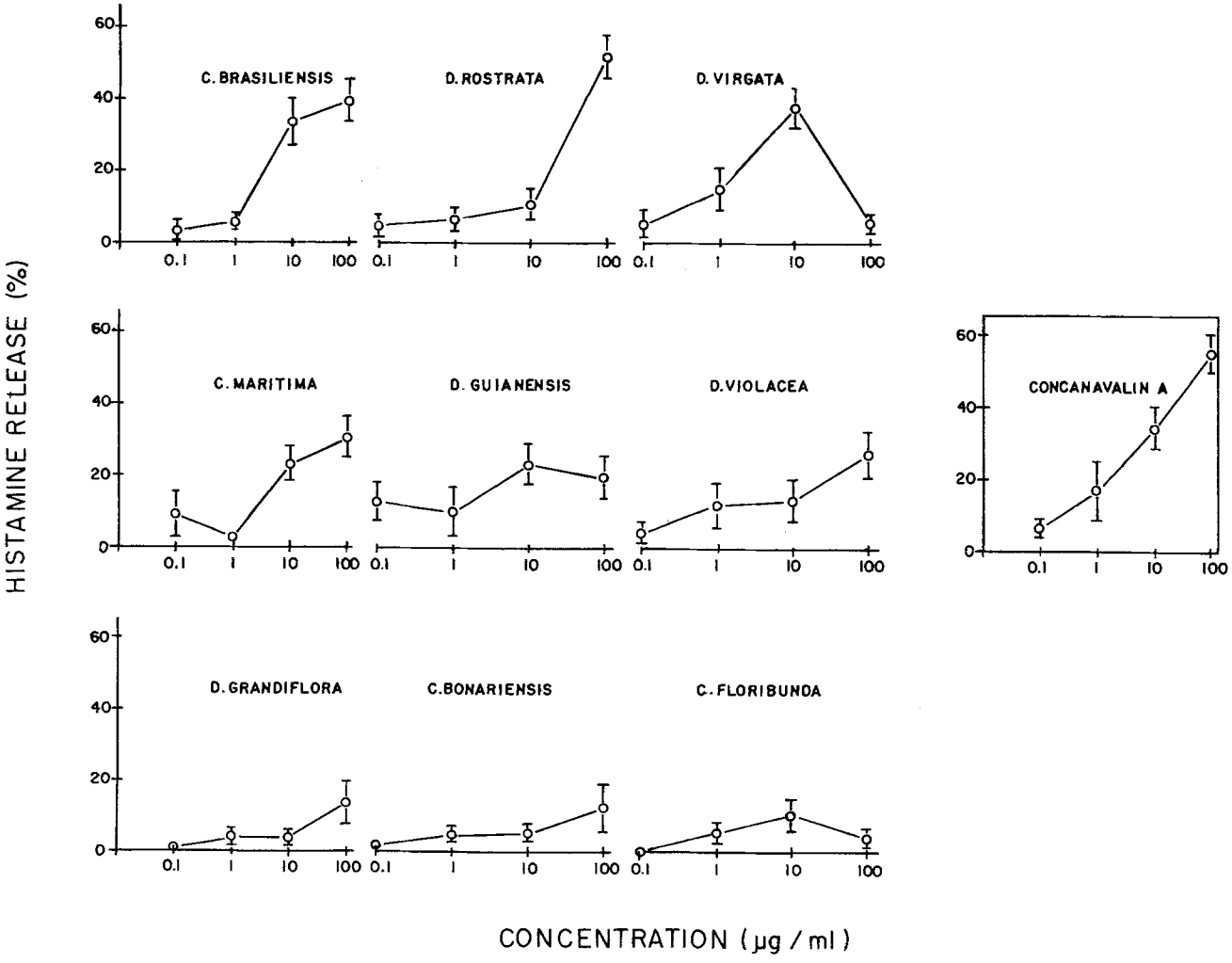

Fig. 1. Dose–response curves of the histamine release induced in RPMC by lectins from Brazilian beans. Comparison with the effect of concanavalin A. The symbols are means of seven experiments and the bars the SEM.

Results

Histamine release induced by the lectins in RPMC

The lectins from *C. brasiliensis*, *D. rostrata*, and *D. virgata* produce histamine release in RPMC with the potency and efficacy similar to concanavalin A. The histamine release induced by the lectin from *D. virgata* increases till 10 μg/ml and decreases with higher doses. The lectin from *D. violacea* is less potent than concanavalin A, although the maximum effect was not reached with the higher dose used. The lectins from *C. maritima* and *D. guianensis* have lower potency and efficacy than concanavalin A. The lectins from *D. grandiflora*, *C. bonariensis* and *C. floribunda* produced a very low level of histamine release (Fig. 1).

Effect of increasing concentration of calcium on histamine release induced by the lectin from D. virgata

Figure 2 shows the histamine release induced in RPMC by the lectin from *D. virgata* in the presence of different concentrations of calcium. It can be seen that the decrease in the percentage of histamine release caused by a high dose of the lectin from *D. virgata* can be abrogated in dose–response curves where the calcium concentration is increased. This is already verified with 3 m*M* calcium. It is also observed that the histamine released increases more than two times comparing the calcium concentrations of 1 and 10 m*M* despite the lectin doses.

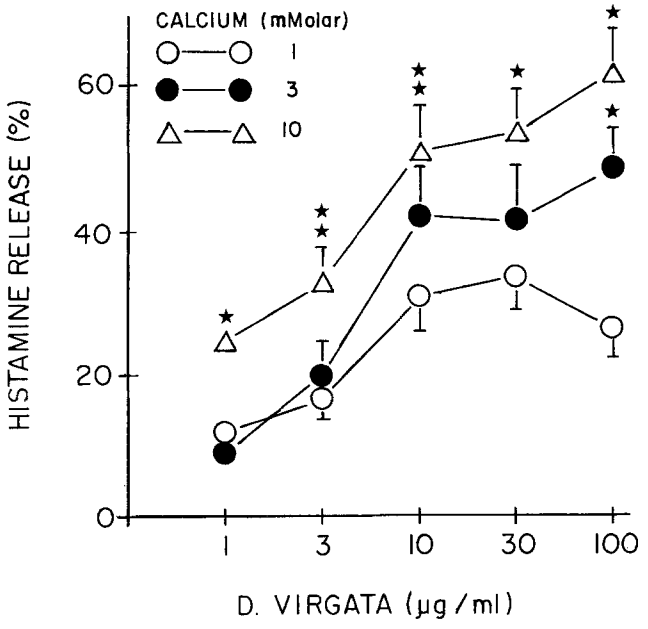

Fig. 2. Dose–response curves of the histamine release induced in RPMC by the lectin from *D. virgata* in the presence of different concentrations of calcium. The symbols are means of seven experiments and the bars the SEM. The means statistically different from the correspondent values obtained with calcium 1 m*M* are indicated. (*) $P<0.005$; (**) $0.025>P>0.01$.

Discussion

Most of the lectins studied are able to release histamine from RPMC. Nevertheless, they strongly differ in potency and efficacy. Although the lectins studied belong to the same tribe and show very high homology (80–90%) with respect to their primary structures, differences among them have also been observed in human lymphocyte stimulation and interferon gamma production by peripheral mononuclear cells in humans [16]. These differences might be explained by different fine affinity sites of the lectins that bind to the targets that trigger the biological effects. In addition, the influence of the proportion between the intact and fragmented subunits which are known to take part in the fine structures of the agglutin isoforms as seen for concanavalin A [15], *D. grandiflora* [10], and *D. guianensis* [13] lectins could not be excluded.

The lectin from *C. brasiliensis* induces histamine release in peritoneal, pleural and check pouch mast cells from hamster [17] in a pattern similar to that observed for RPMC, suggesting similar mechanism. On the other hand, the lectin from *D. grandiflora* is reported to induce mouse mast cells degranulation with a dose of 50 μg/ml [18] that probably induces a negligible histamine release in RPMC (in our experiments 100 μg/ml releases less than 20%). The difference could be due to the result presentation as a morphological data instead of the amount of histamine released. This difference might also be explained by the functional heterogeneity that has been pointed out for mast cells from different species [19].

The histamine release induced by the lectin from *D. virgata* shows a dose–response curve with a bell shape form that characterizes an inhibition caused by the high doses. This peculiarity is also reported for concanavalin A-induced histamine release in rat and mouse mast cells, guinea-pig and rabbit leukocytes [8], hamster mast cells [20], and human leukocytes [8, 21]. The histamine release induced by concanavalin A is a calcium-dependent phenomenon [8, 22] as also are all other histamine release inducers. Besides sugars and manganese, concanavalin A binds to calcium [2], a characteristic that is shared by the lectin from *D. virgata* and other *Diocleineae* lectins [14]. Our results showed that the quenching of the histamine release observed with high doses of the lectin from *D. virgata* can be reversed by increasing the concentration of calcium. Besides, even the histamine release induced by low concentration is potentiated by calcium 10 m*M*. This fact suggests that the quenching observed with high doses of the lectin from *D. virgata*, and that reported for concanavalin A, are due to the sequestration of the calcium needed for the histamine release.

We concluded that like concanavalin A, the lectins from *C. brasiliensis*, *D. rostrata*, and *D. virgata* showed potency and efficacy enough to be used in studies about histamine release. Besides, our results suggest that lectins with very similar physicochemical properties can act differently toward the same biological system, probably due to differences in the fine sugar specificity.

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