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INFLUENCE OF THE SYNTHETIC
PENTAPEPTIDE $\text{LAsp-Ser-Asp-Pro-Arg}$
ON THE DIRECT AND INDIRECT MAST CELL
DEGRANULATION INDUCED BY
REAGINIC ANTIBODIES

By

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The Prausnitz-Kustner (PK) reaction in man is a known method for proving the presence of reagin IgE antibodies towards various allergens (7). During recent years a method has been devised for detection of IgE-type antibodies *in vitro* either in human patients or experimental animals. This method is based on mast cell degranulation test in a direct way by using cells from the sensitized individual (9,11) or in the indirect form by employing normal rat mast cells (8,10,11).

It was claimed recently (3) that a synthetic pentapeptide can inhibit the PK reaction and that this effect is due to similarity in structure with the specific IgE binding site for the mast cells (2). The amino acid sequence of the pentapeptide is $\text{LAsp-Ser-Asp-Pro-Arg}$ (3).

The present work was started in order to find out whether the synthetic pentapeptide can also inhibit the *in vitro* mast cell degranulation characteristic for the reaction between suitable antigen and IgE bound to mast cells. For this pur-

pose, rat mast cells were used either in a direct or indirect degranulation test. The results obtained support the assumption that this synthetic pentapeptide can interfere with the interaction between the bound IgE antibody and the corresponding antigen.

MATERIAL AND METHODS

Animals: Adult randomly bred albino rats, 150–200 g in weight of Charles River strain obtained from a local breeding colony, were used.

Test compounds: Human gama globulin (HGG, Magen David Adom Israel Blood Service) and egg albumin (EA, crystallized and lyophilized preparation, Sigma, U.S.A.), were used for sensitization of rats. Human serum was obtained from normal individuals and from a patient found sensitive to erythromycin (1). Erythromycin (Er) used for the *in vitro* test was purchased from Abbott Lab. U.S.A. The pentapeptide (PP) LAsp-Ser-Asp-Pro-Arg (M.W 596) was kindly prepared and provided by Dr. M. Fridkin, Dept. of Organic Chemistry, the Weizmann Institute of Science, Rehovot. The compound was dissolved in PBS, pH 7.4, before use.

Inoculations: An initial group of 71 rats were injected into the hind foot pads with 12.5 mg HGG emulsified in an equal amount of complete Freund's adjuvant (CFA, Difco) in a total volume of 0.4 ml per animal. A group of 60 animals out of 71 was divided into five subgroups: six animals were injected 5 days later with 1 mg PP/ml intraperitoneally (i.p.); another five animals received on the same day an injection of 1 ml PBS i.p.; a subgroup of 25 rats were injected i.p with 1 mg/ml PP 12 days after the HGG inoculation; other 22 rats received i.p PBS instead of PP at the same time; the remaining two rats were used for the mast cell degranulation test, without any intermediate treatment, on the 12th day after sensitization with HGG.

A subgroup of 11 animals received a second injection of the same quantity of HGG in CFA intramuscularly (i.m), 9 days after the first inoculation. Three days afterwards, five out of 11 animals were injected i.p with 1 mg PP/ml. The other six animals were injected with 1 ml PBS i.p. A small group comprising 15 rats were sensitized with 20 mg EA per animal emulsified in CFA by injection into the hind foot pads. Seven rats were injected i.p 12 days afterwards with 1 mg/ml of PP; a further six rats received PBS instead of PP. The remaining two rats were used directly for the mast cell degranulation test.

In all cases the animals were sacrificed 18 h after the i.p treatment (PP or PBS) and the peritoneal mast cells were used for the direct mast cell degranulation test (DMCD).

Direct mast cell degranulation (DMCD) test: The method employed has been described previously (9,11). Peritoneal mast cells suspended in M 199 (5), were

incubated *in vitro* at 37° C for 20 min with the sensitizing antigen in optimal concentration, as determined in preliminary tests (6.25 mg HGG or 0.5 mg EA). The percentage of degranulated mast cells out of 100 cells was determined by microscopic examination on a slide stained with neutral red (8). Mast cells from rats which received *in vivo* treatment with PP were put in contact *in vitro* with the antigen alone. Cells originating from rats untreated with PP were used either as controls or for determination of inhibition *in vitro* of DMCD by PP.

The inhibition test was carried out by incubating at room temperature a suspension of 2 ml (2.6×10^5) mast cells with PP at a final concentration of 50 μ g/suspension. The treated cells (0.05 ml) were brought in contact with the antigen (0.05 ml), either immediately afterwards or 15 min later, and incubated as usual at 37° C for 20 min. Mast cells incubated without antigen were used as controls.

Indirect mast cell degranulation (IMCD) test: The test was performed as previously described (8). Mast cells of normal rats were incubated with sera of rats sensitized with HGG, EA or with human serum from a patient allergic to erythromycin (Er) and with the corresponding antigens (HGG, EA, Er). The controls included either normal serum or antigen only, in absence of serum. The incubation mixture contained 0.05 ml serum undiluted, 0.05 ml mast cell (1.3×10^5 /ml) suspension and 0.05 ml antigen (6.25 mg HGG, 0.5 mg EA or 15 μ g Er). The readings of degree of degranulation were done after incubation at 37° C for 20 min. The inhibitory effect of PP was evaluated by preincubating the mast cells (1.3×10^5 /ml) with a final concentration of 50 mg PP per 2 ml cell suspension at room temperature. The other ingredients (serum and antigen) were added immediately afterwards. The counts were done as previously described. Controls of normal mast cells with PP and with antigen or serum only, were included.

RESULTS

Effect of PP on DMCD: The effect of the PP on the percentage of degranulated mast cells in DMCD tests was estimated under two conditions: by addition of PP *in vitro* either simultaneously with the antigen or 15 min before the antigen addition to the mast cells, or by injecting PP to the sensitized rats 18 h before withdrawal of cells. Addition of PP (50 μ g/2ml) *in vitro* resulted in decrease in the percentage of degranulated mast cells in the presence of the antigen. This effect was evident in the case of both antigens (HGG or EA) and was more marked when the PP was added to

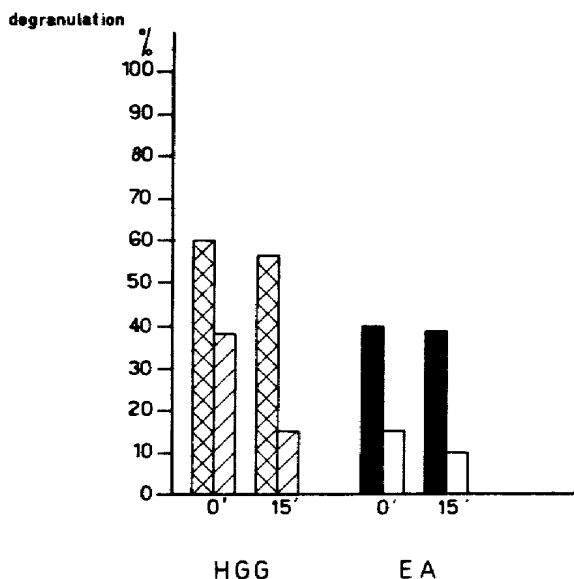


Fig. 1.

Effect of pentapeptide *in vitro* on direct mast cell degranulation (DMCD) test. Mast cells from rats inoculated with HGG:

- ☒ Untreated with PP before adding the antigen.
- ☑ Treated with PP before adding the antigen.

Mast cells from rats inoculated with EA:

- Untreated with PP before adding the antigen.
- Treated with PP before adding the antigen.

0'; 15' Incubation time of PP with mast cells before adding the antigen.

the mast cells 15 min before the antigen. The results are presented in Fig. 1.

The effect of the *in vivo* treatment with the PP on DMCD was also evaluated. The PP was injected on the 12th day after inoculation of the antigen. In the case of both antigens (HGG or EA) the degree of DMCD was markedly lower

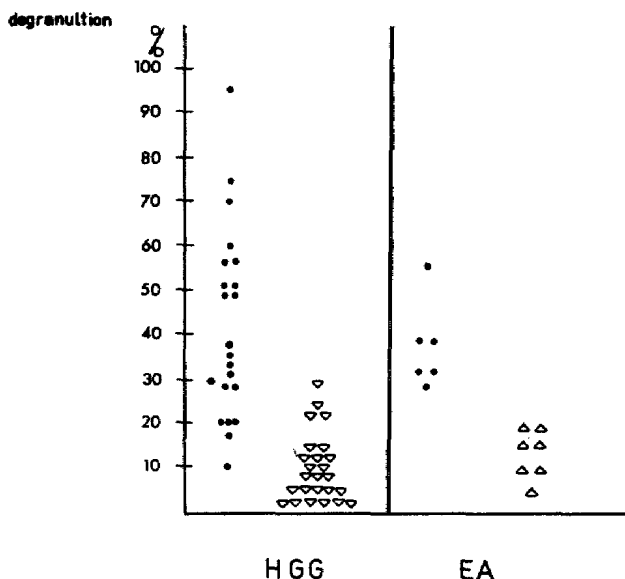


Fig. 2.

Inhibition of direct mast cell degranulation (DMCD) by injecting the penta-peptide intraperitoneally into sensitized (12 days) rats.

DMCD test with mast cells from:

- rats inoculated i.p. with PBS 18 h before the test.
- △ rats inoculated i.p. with PP 18 h before the test.

with mast cells originating from animals treated with the PP (Fig. 2).

The effect of PP treatment was also evaluated with mast cells taken on the 5th or 7th day after one inoculation of HGG and from rats receiving a second injection of HGG on the 9th day after the first one and tested 12 days after the first inoculation. Mast cells taken both from PP-treated and untreated rats on the 5th or 7th day after the antigen inoculation showed a low degree of degranulation. Treatment with PP resulted in decrease in percentage of degranulated

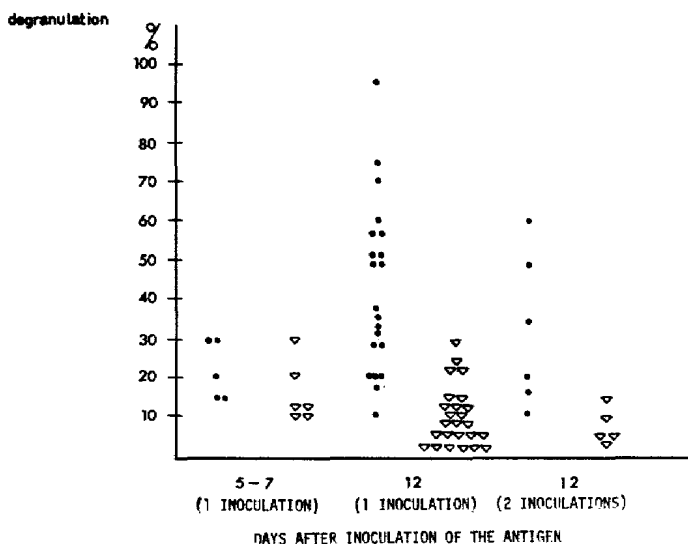


Fig. 3.

Inhibition of direct mast cell degranulation (DMCD) test by pentapeptide injected intraperitoneally at different days after antigen inoculation (antigen-HGG).

DMCD test with mast cells from:

- rats inoculated i.p. with PBS 18 h before the test.
- ▼ rats inoculated i.p. with PP 18 h before the test.

mast cells obtained from rats sensitized with two injections of HGG. The results are presented in Fig. 3.

Effect of PP on the IMCD: Inhibition of IMCD test by PP was observed in the two systems employed. In the first one, PP was added to normal rat mast cells treated with anti-HGG serum or anti-EA and the corresponding antigen. In this case full inhibition of degranulation was observed. In the second case, addition of PP to mast cells sensitized with serum from a person allergic to Er resulted in drop of percent of degranulated cells, from 36 percent to 2 percent. The results are shown in Fig. 4.

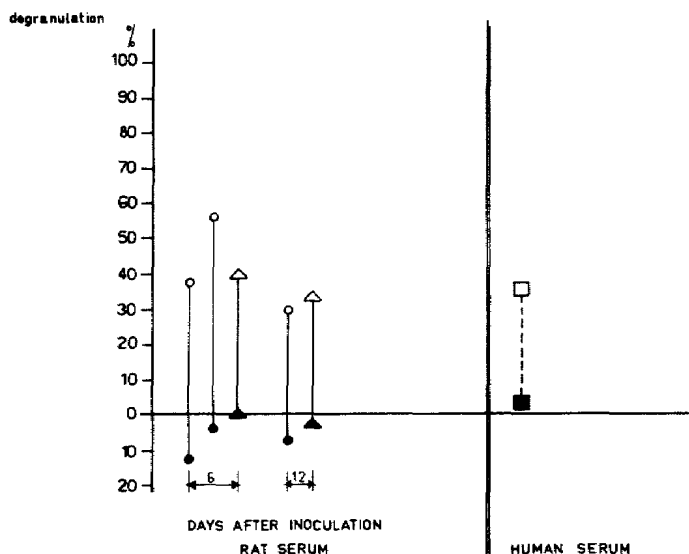


Fig. 4.

Effect of pentapeptide on indirect mast cell degranulation (IMCD) test.

- rat anti-HGG serum + HGG
- rat anti-HGG serum + HGG + PP
- △ rat anti-EA serum + EA
- ▲ rat anti-EA serum + EA + PP
- serum from human allergic to Er + Er
- serum from human allergic to Er + Er + PP

DISCUSSION

The main finding is that the pentapeptide (PP) $LAsp-Ser-Asp-Pro-Arg$ prevented degranulation of mast cells both in DMCD and IMCD test.

It is assumed that the DMCD test reflects interaction between antibody bound to the mast cell, presumably IgE (4), and the corresponding allergen. The inhibitory effect of PP in this system would indicate that this compound prevents the interaction between the antigen and the already

bound antibody. The main difference between the DMCD and the IMCD test is that in the latter test reagins reacting with a certain antigen attach themselves to normal mast cells. Therefore, it seems plausible that the inhibitory effect of PP in this system is due to prevention of binding of sensitizing antibodies to the mast cells.

It was assumed that the main type of antibodies involved in the test of mast cell degranulation is IgE (4,6). Moreover, it was claimed that inhibition of Prausnitz-Kustner reaction by a pentapeptide of identical composition is due to the fact that this compound represents the structure of the specific IgE binding site for the mast cells (3).

It is of interest to mention that PP was effective also when given *in vivo* to sensitized rats. This finding suggests that PP may have a certain affinity for mast cells *in vivo*. Therefore it might be feasible to use suitable synthetic peptides for the therapy of allergic states in view of their ability to prevent the interaction between reagins and corresponding allergens. The use of *in vitro* tests might be valuable in order to evaluate the importance of various parameters (chemical structure, optimal concentration, incubation time, etc.) concerned with inhibition of interaction between reaginic antibodies and corresponding allergens by synthetic compounds.

SUMMARY

The influence of the synthetic pentapeptide LAsp-Ser-Asp-Pro-Arg on mast cell degranulation induced by reaginic antibodies was evaluated by the direct and indirect degranulation test.

Treatment with the synthetic compound resulted in inhibition of both direct mast cell degranulation and indirect degranulation.

Inhibition of the direct mast cell degranulation was observed when the peptide was injected intraperitoneally into rats sensitized with either human gamma globulin (HGG)

or egg albumin (EA) before withdrawing the mast cells. Decrease in the percentage of degranulated cells was also observed following exposure of the cells from sensitized animals to the peptide *in vitro*. The inhibition effect was most marked in cell suspensions taken on the 12th day after sensitization, especially if the animals were sensitized with two inoculations of HGG.

The indirect degranulation test was performed with normal rat mast cells treated with either serum from a patient sensitive to erythromycin (Er) or sera from rats sensitive to HGG or EA and exposed to the corresponding antigen. Addition of the synthetic peptide *in vitro* to the reaction mixture resulted in inhibition of degranulation.

It is concluded that the pentapeptide tested was able to effect either the interaction between the already bound reaginic antibody and the corresponding antigen, or to prevent binding of these antibodies to normal mast cells.

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