

The Anti-Anaphylactic Action of Tiaramide Hydrochloride

M. L. RENOUX, B. J. WEILL, D. WALLACH and E. CHWETZOFF

Laboratoire de Pathologie Expérimentale et d'Immunologie Générale Faculté Cochin, Paris, France

Tiaramide hydrochloride (THC) is a new basic, non-steroidal, anti-inflammatory drug. Its anti-anaphylactic action has been investigated using rat mast cells. It was found that THC exerts a strong inhibitory action on antigen-induced and compound 48/80-induced histamine release from rat peritoneal mast cells in a fluorometric assay. Compound 48/80-induced vasodilatation in rat skin is inhibited by prior intradermal injection of THC, as measured by blueing of skin due to intravascular Evans blue dye. THC also inhibits radio-labeled serotonin release from compound 48/80-challenged rat mast cells.

In these experimental systems a similar action was exerted by disodium cromoglycate, but higher drug concentrations were needed.

Further studies are needed to determine the exact mode of action of this drug and its eventual clinical use in the field of allergic diseases.

Key words: anti-anaphylactic drugs; anti-inflammatory drugs; compound 48/80; histamine release; rat mast cells; serotonin release.

Accepted for publication 29 November 1977

Drugs which inhibit mast cell degranulation are effective in the treatment of allergic diseases, and particularly bronchial asthma. Disodium cromoglycate (DSCG) is one of the most widely used inhibitors of mast cell degranulation, and its mode of action has been extensively investigated (9). It has proved effective in allergic as well as non-allergic (exercise-induced (3)) asthma. DSCG, which is not absorbed by the digestive tract, must be locally applied, i.e. inhaled, to prevent bronchial mast cell degranulation. Numerous investigators are now looking for new anti-allergic agents, which can be administered orally, with an efficacy similar to that of DSCG, without any side effects.

Tiaramide hydrochloride, 4-((5-chloro-2-oxo-3-benzothiazolyl)acetyl)-1-piperazine ethanol hydrochloride, is a new basic, non-steroidal anti-inflammatory drug which has been recently synthesized (12) (Fig. 1). Pharmacological studies revealed potent in-

hibitory activity on acute and subacute inflammation: inhibition of inflammatory edema, decrease in permeability of capillary vessels, and inhibition of inflammatory granuloma formation. This anti-inflammatory action appeared to be partly due to an inhibitory activity on the release of histamine and other chemical mediators by the mast cells. It was suggested that this drug might be useful in the control of allergic manifestations.

The present work has been undertaken to evaluate the action of tiaramide

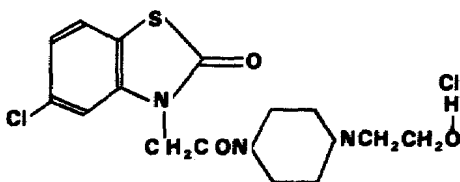


Fig. 1. Tiaramide hydrochloride. M.W. = 392.3.

hydrochloride on rat mast cell degranulation. Three different experimental methods were used, and the action of DSCG compared with that of THC.

MATERIAL AND METHODS

Wistar rats (Charles River, France) weighing between 125 and 250 g were used throughout this study. Only females were used in the immunization experiments.

Histamine release in vitro

Rats were killed by cervical blow. Mast cells were collected by washing the peritoneal cavity with 10 ml of Tris-ACM medium (Tris (hydroxymethyl) aminomethane 25 mM at pH 7.6, Na Cl 0.12 M, KCl 5 mM, CaCl_2 0.6 mM, MgCl_2 1.1 mM, human serum albumin 0.3 mg/ml and glucose 1 mg/ml). The peritoneal cell suspension contained about 10^5 mast cells per ml. THC (Fujizawa Pharmaceutical Co., Osaka, Japan) and DSCG (Fisons S.A., Saint-Denis, France) were dissolved in saline and added at various concentrations to mast cell suspensions. These suspensions were then incubated for 10 min at 37°C, before the addition of the degranulating agent.

Two types of mast cell degranulation were investigated:

1) Non-specific histamine release was studied by submitting mast cell suspensions from normal rats to the action of the degranulating agent, compound 48/80 (Wellcome, Brussels), at final concentrations of 0.5 $\mu\text{g/ml}$ and 0.75 $\mu\text{g/ml}$ for 10 min at 37°C. Preliminary assays had shown that these concentrations achieved inframaximal histamine release, particularly suitable for inhibition studies (Fig. 2).

2) Anaphylactic histamine release. Rats used in these experiments were immunized by intramuscular injection of 0.2 ml of crude egg white and 0.5 ml of *Bordetella pertussis* vaccine containing $15 \cdot 10^9$ killed organisms (Vaxicoq adsorbé, Institut Mérieux, Lyon); mast cells were collected on the 15th day

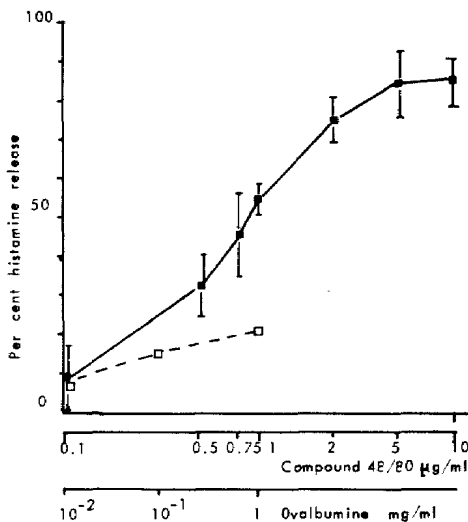


Fig. 2. *In vitro* histamine release by peritoneal mast cells. $\blacksquare$ — $\blacksquare$, per cent of histamine released upon action of compound 48/80: mean value of 10 determinations $\pm$ s.e.m. 0.5 and 0.75 μg concentrations were selected for the present work.

$\square$ — $\square$, per cent of histamine released upon action of antigen on mast cells from sensitized animals; in view of the diversity of the dose-response curves in this system, it was considered preferable to show the results of a typical experiment.

after antigen injection. Suspensions of peritoneal mast cells from sensitized rats were challenged with a solution of crystallized ovalbumin in saline at final concentrations of 0.01, 0.1, and 1 mg/ml, and incubated for 10 min at 37°C.

Histamine contained in the supernatants obtained after centrifugation in cold was assayed by a modification of the technique described by May et al. (5), using orthophthaldehyde. Fluorescence measurements were performed with a Perkin-Elmer 1000 fluorometer, and the results automatically recorded. The results were expressed in percent of total histamine released by an aliquot of mast cell suspension heated at 80°C for 5 min, minus the histamine content of control tubes without histamine releaser. Two supplementary

controls were included in each experiment: controls with the test substance alone (without histamine releaser) and controls of maximal mast cell reactivity with compound 48/80 at 100 $\mu\text{g/ml}$, a concentration inducing maximal histamine release.

In vitro labeled serotonin release

It has been demonstrated that rat mast cells can incorporate exogenous serotonin *in vitro* (6). After incorporation of ^{14}C -labeled serotonin, the kinetics of histamine and serotonin release are identical when mast cells are challenged by a degranulating agent such as compound 48/80. The use of radio-labeled mediators represents a very sensitive method for studying mast cell degranulation.

Mast cells were collected from peritoneal washings as indicated above. Aliquots containing 10^5 cells were incubated at 37°C for 45 min with 0.05 microcurie of ^{14}C serotonin (48 mCi/mM) (CEA, France). Mast cells were washed twice in cold, the suspension reconstituted to the same concentration and incubated for 7 min with the test substance at various concentrations. Compound 48/80 (0.75 or 0.50 μg) was then added to each aliquot. After 15 min centrifugation at 500 g, the release of labeled serotonin was measured in the supernatants. ^{14}C serotonin was assayed with a Packard spectrometer using a ready-to-use scintillation system (Instagel, Packard Instrument Company Inc.).

Inhibition of anaphylactic-like vasodilatation in vivo

Intradermal injection of compound 48/80 induces a strong vasodilatation due to the vaso-active amines released by mast cell degranulation. When Evans blue is injected into the blood stream, its extravasation helps objectivize this dermal vasodilatation, in the same way as with passive cutaneous anaphylaxis techniques.

0.1 ml of a dilution (from 10^{-2} M to 10^{-7} M) of the test substance was injected into the shaved dorsal skin of a normal rat. Saline

was injected at another site, as control. Ten minutes later, 0.05 ml of compound 48/80 (100 $\mu\text{g/ml}$) was injected into the same sites, and 1 ml of a solution of Evans blue dye (0.25%) was injected by cardiac puncture.

Twenty minutes later the animal was killed and the diameter of the blue spots measured at the inner surface of the skin. Inhibition of blueing due to the test substance is expressed by comparison with the control spot.

RESULTS

Action of THC on histamine release by rat mast cells

Results of histamine release experiments, expressed in percentages of inhibition are shown in Figs. 3 and 4. THC alone was tested on anaphylactic histamine release; at high concentrations (10^{-3} M) the drug inhibits the anaphylactic reaction, irrespective of the antigenic challenge. At lower concentrations (10^{-5} M and 10^{-7} M) histamine release is only partly inhibited. THC proved effective in inhibiting compound 48/80-induced histamine release at 10^{-3} M and 10^{-5} M concentrations, but not at 10^{-7} M. A comparative study of the action of DSCG shows that at the same

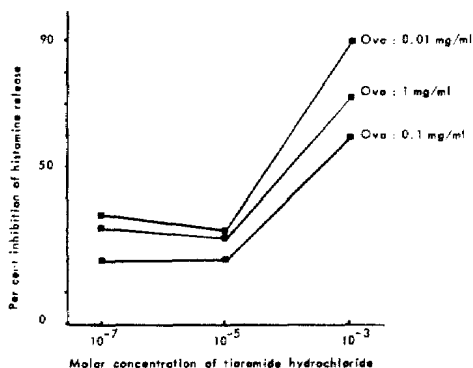


Fig. 3. Inhibition of anaphylactic histamine release by THC. Compiled from results in five experimental animals. S.e.m. was not calculated because of the small number of animals in this group.

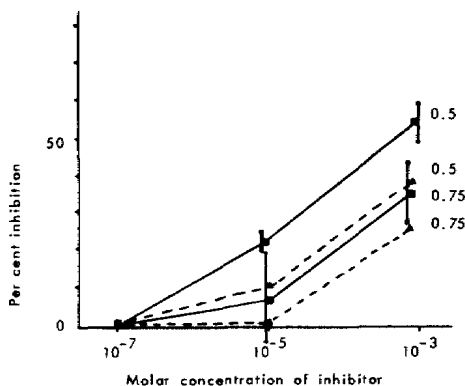


Fig. 4. Inhibition of compound 48/80-induced histamine release by THC (—) and DSCG (---). Compiled from results in eight experimental animals. 0.5 and 0.75, concentrations of compound 48/80 (μ g/ml). Standard deviation indicated.

molar concentrations a stronger inhibitory effect is achieved by THC. Nevertheless, a better inhibitory action is exerted by THC when the histamine releasing stimulus is weaker, as shown by the results obtained with two different concentrations of compound 48/80, and with anaphylactic histamine release. In the latter case, the average percentage of histamine release was clearly lower than after challenge by compound 48/80, and the inhibition by THC was more potent.

Action of THC on compound 48/80-induced labeled serotonin release

Mast cells having incorporated labeled serotonin release this mediator during the process of degranulation. In the presence of THC at a concentration of 10^{-2} M, this release is almost completely inhibited; the inhibition of serotonin release is dose-dependent and lower concentrations gave a much smaller reaction. Percentages of inhibition are shown in Fig. 5. Spontaneous degranulation was taken into account when calculating the percentages of inhibition. DSCG can also inhibit radio-labeled serotonin release. At a concentration of 10^{-4}

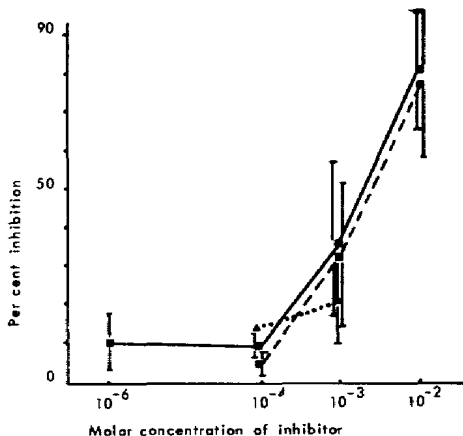


Fig. 5. Inhibition of 48/80-induced ^{14} C serotonin release.

Mast cells were challenged with compound 48/80 at the concentrations of 0.50 μ g/ml (—), and 0.75 μ g/ml (— and ---) in the presence of THC (■—■ and □—□), or DSCG (▲...▲). Mean of six to eight experiments. Bars indicate $\pm$ s.e.m.

M, THC and DSCG act similarly. However, at a 10^{-3} M concentration, DSCG is less active than THC. The experiments being focused on the inhibitory activity of THC compared with DSCG in the therapeutic range of concentrations, the inhibitory action of 10^{-2} M DSCG was not tested.

It must be noted that THC at high concentrations (10^{-2} M) has a slight degranulating action in the absence of compound 48/80. This effect was not observed with DSCG.

Inhibition of compound 48/80-induced vasodilatation

This experimental process allowed us to demonstrate the clear action of anti-allergic substances tested on dermal vasodilatation. When THC is highly concentrated *in situ*, it completely inhibits the otherwise potent action of intradermally injected compound 48/80. DSCG seems to be less active, as can be seen in Fig. 6. In such *in vivo* experiments, the whole phenomenon of immediate hypersensitivity is implicated, including mast

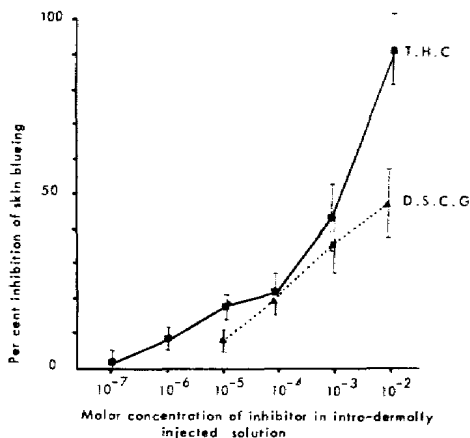


Fig. 6. Inhibition of compound 48/80-induced vasodilatation by THC (■—■) and DSCG (▲·····▲). Mean of six to ten experiments. Bars indicate $\pm$ s.d.

cell degranulation as well as the physiologic effects of mediators.

DISCUSSION

Specific mediator release induced by antigen-antibody combination is obviously the main feature of allergic diseases. However, non-specific mast cell degranulation is likely to play a part in frequent pathological conditions such as exercise-induced asthma, intrinsic asthma, or non-allergic urticaria. The release of vaso-active amines by mast cells is then included in the complex events of acute inflammatory processes. In such cases, mediator release seems to be independent of immunologic mechanisms. The experimental study of non-specific, compound 48/80-induced mast cell reactivity thus appears to be a useful tool for the evaluation of drugs active in these fields. It is generally accepted that the release of chemical mediators induced by 48/80 does not specifically differ from the sequence of activation following the interaction between cell-bound antibody and the specific antigen; both processes act selectively on mast cells,

and their action requires energy. The first reports about DSCG (2) had denied any effect of this drug on the skin lesions induced by compound 48/80. However, later findings by the same author (8) clearly indicate that DSCG also inhibits the action of this non-specific histamine releaser. Our results confirm this finding and the value of such tests for evaluating new anti-allergic drugs.

Comparison between DSCG and THC shows that the same degree of action can be obtained with both drugs, but, in the different experimental systems tested, the molar concentration of DSCG must be higher than that of THC. The value of this finding seems to be confirmed by the fact that our results concerning DSCG are comparable to those found in other reports based on the study of similar systems. This does not imply that both drugs are acting at the same stage of mast cell degranulation: in fact the exact mechanism of action of DSCG and other anti-allergic compounds still remains controversial (1, 4, 10, 13).

Such results, clearly indicating an inhibitory activity on mast cell degranulation, suggest that THC might be useful in the management of allergic diseases. One must also consider that this drug is quickly absorbed after oral administration (7), and is well tolerated, as experienced when it is used as an anti-inflammatory drug. In fact, a trial in asthmatic children has been performed, and encouraging preliminary results have been recently reported (11).

REFERENCES

1. Coulson, C. J., Ford, R. E., Marshall, S., Walker, J. L., Wooldridge, K. R. H., Bowden, K. & Coombs, T. J.: Interrelationship of cyclic nucleotides and anaphylactic reactions. *Nature* 265, 545-547, 1977.
2. Cox, J. S. G.: Disodium cromoglycate (FPL 670) (Intal): a specific inhibitor of reaginic antibody-antigen mechanisms. *Nature* 216, 1528-1529, 1967.
3. Davies, S. E.: Effect of disodium cromoglycate on exercise-induced asthma. *Br. Med. J.* 3, 593-594, 1968.

4. Foreman, J. C., Hallett, M. B. & Mongar, J. L.: Site of action of the antiallergic drugs cromoglycate and doxantrazole. *Br. J. Pharmacol.* **59**, 473P-474P, 1977.
5. May, C. D., Lyman, M., Alberto, R. & Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *J. Allergy* **46**, 12-20, 1970.
6. Morrison, D. C., Roser, J. E., Henson, P. M. & Cochrane, C. G.: Activation of rat mast cells by low molecular weight stimuli. *J. Immunol.* **112**, 573-582, 1974.
7. Noda, K., Noguchi, H., Okui, M., Tada, K., Morimoto, Y. & Kozatani, J.: Absorption, distribution, excretion and metabolism of Tiamamide hydrochloride: a new anti-inflammatory drug. *Arzneim. Forsch. (Drug Res.)* **22**, 732-743, 1972.
8. Orr, T. S. C., Hall, D. E., Gwilliam, J. M. & Cox, J. S. G.: The effect of disodium cromoglycate on the release of histamine and degranulation of rat mast cells induced by compound 48/80. *Life Sci.* **10**, 805-812, 1971.
9. Orr, T. S. C.: Recent developments concerning the mast cell and the mode of action of disodium cromoglycate. *Acta Allergol.* **30**, Suppl. XII, 13-32, 1975.
10. Roy, A. C. & Warren, B. T.: Inhibition of cAMP phosphodiesterase by disodium cromoglycate. *Biochem. Pharmacol.* **23**, 917-920, 1974.
11. Sasaki, S., Motonoga, M. & Taniguchi, K.: Clinical effect of tiamamide on children with bronchial asthma. IXth. International congress of allergology, Oct. 24-30, Buenos-Aires, 1976.
12. Takashima, T., Kadoh, Y. & Kumada, S.: Pharmacological investigations of benzothiazoline derivatives. *Arzneim. Forsch. (Drug Res.)* **22**, 711-715, 1972.
13. Tateson, J. E. & Trist, D. G.: Inhibition of adenosine 3'5' cyclic monophosphate phosphodiesterase by potential antiallergic compounds. *Life Sci.* **18**, 153-162, 1976.

Address:

Prof. Mario-Louis Renoux

Laboratoire d'Immunologie et de Médecine Expérimentale

U.E.R. Cochin Port-Royal

24, rue du Faubourg Saint-Jacques

75674-Paris Cedex 14

France

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.