Rats are commonly used in anaphylaxis models, mainly in intestinal anaphylaxis. Hypersensitivity mechanisms are complex and they are not clearly defined. Ovalbumin (OVA) is commonly used for studies on the hypersensitivity mechanism. However, the potential pro-inflammatory mediators induced by this antigen in the model of paw oedema in immunized rats are still not completely understood. This work examines the pharmacological modulation of several mediators involved in rat hind paw immune oedema induced by OVA. Wistar rats were previously immunized (14–18 days) with OVA (30 μg, intraperitoneally) or sham-sensitized with aluminum hydroxide (control). The paw volumes were measured before the antigenic stimuli and 1, 2, 3 and 4 h after the intraplantar injection of OVA (10 μg/paw). Subcutaneous injection of dexamethasone, diphenhydramine, cyproheptadine, chlorpromazine or methysergide significantly inhibited \( p < 0.05 \) the allergic paw oedema. The dual inhibitor of cyclooxygenase and lipoxygenase (NDGA), the cyclooxygenase inhibitor (indomethacin), the lipoxygenase inhibitor (MK-886), the PAF antagonist (WEB 2086), the mast cell stabilizer (ketotifen), and the anti-histamine (meclizine) did not inhibit the immune oedema. In addition, thalidomide and pentoxyfylline (anti-tumour necrosis factor drugs) were ineffective against OVA-induced oedema. The fact that indomethacin, MK-886, NDGA and WEB 2086 are unable to inhibit this allergic oedema indicates that the dexamethasone action seems not to be via phospholipase A2, but possibly due to the synthesis and/or the inhibitory activity of cytokines. The paw oedema inhibition by diphenhydramine, but not by meclizine, may suggest a different mechanism, which is independent of the effect of histamine. These data indicate that allergic oedema is more sensitive to anti-serotonin drugs, mainly anti-5-HT2, suggesting that the principal mediator of this inflammatory response is serotonin.

**Key words:** Rat paw immune oedema, Anti-inflammatory drug, Anti-allergic drug, Ovalbumin

**Introduction**

Allergic processes are complex disorders in which inflammatory and immunological mechanisms are involved. One of the most important approaches used in the examination of the immunopathological mechanisms of anaphylactic and inflammatory disorders is to elicit the formation of paw oedema, injecting various substances into the subplantar tissue of rats or mice. \(^1\) Inflammation often occurs after a subplantar injection of a number of substances in the hind paw of rats or mice. Many anti-inflammatory drugs have been tested for their ability to inhibit hind paw oedema.

The vasoactive amines, histamine and serotonin, play a crucial role in type I hypersensitivity. \(^2\) The most important vasoactive mediators that are stored in mast cells and basophil granules are histamine in humans, as well as serotonin (5-hydroxytryptamine) in rodents. Histamine was one of the first inflammatory mediators thought to be important in the pathophysiology of asthma. \(^5\) Like histamine, serotonin is also capable of increasing vascular permeability, of dilating capillaries and of producing the contraction of non-vascular smooth muscles. Most serotonin is stored in the gastrointestinal tract and the central nervous system, but a large amount is also stored in the dense granules of platelets. \(^6\)

Rats are commonly used in the study of anaphylaxis, particularly those involving the intestinal tract. \(^7\) Pre-clinical studies are necessary for prospective research on physiopathology of food allergy.
Materials and methods

Animals

Wistar rats (130–230 g body weight) of both sexes were housed in a room with free access to water and food until used. All animals were supplied by Clinical Research Unit, Federal University of Ceará, and the protocol complied with the Occupational Health and Safety in the Care and Use of Research Animals.

Sensitization procedure

OVA was dissolved in phosphate-buffered saline (PBS) and mixed with an equal volume of coloidal aluminium hydroxide, Al(OH)$_3$. The rats were sensitized on day 0 by intraperitoneal (i.p.) injections of 30 mg of OVA/rat dispersed in 0.5 ml of PBS and Al(OH)$_3$. The control rats were injected with an emulsion containing equal volumes of PBS and Al(OH)$_3$.

Quantification of immune paw oedema

Fourteen to 18 days after the injection of antigen to sensitization or Al(OH)$_3$ (sham-sensitization), the animals received an intraplantar injection of 10 mg of OVA in the right hind paw, diluted in 100 μl of PBS. The volume of the hind paw of each animal was measured by plethysmograph (7150 plethysmometer; Ugo Basile, Italy) before the injection of the inflammatory challenge (time 0) and 1, 2, 3 and 4 h after the challenge. The increase in paw volume (∆ volume) was obtained by subtracting the paw volume measured prior to the application of stimuli from the volumes for the different time-points. The results were expressed as the increase in paw volume (ml) calculated by subtracting the basal volume. The area under the time-course curve was also calculated and the results expressed in arbitrary units.\(^9,10\)

Drug modulation of OVA-induced paw oedema in immunized rats

In the present study employing specific antagonists, we investigated the importance of various putative mediators of inflammation in the development of the oedema induced by OVA, in the hindpaw of sensitized rats. The effects of anti-inflammatory, anti-histamine and anti-allergic drugs against anaphylactic paw oedema were studied.

Drug administration

One hour before the intraplantar challenge, animals were treated subcutaneously (s.c.) with inhibitors. The drugs were diluted in sterile PBS. In control animals, sterile PBS replaced the antagonists. Most of the doses used in this study have commonly been shown in the literature to inhibit the corresponding binding sites.\(^4,11,12\)

Systemic depletion of mast cells\(^{13}\)

Animals were pretreated i.p. with compound 48/80 during 4 days (0.6 mg/kg, twice a day for 3 days and 1.2 mg/kg, twice on the 4th day). On the 5th day, the intraplantar challenge was made and the oedema was evaluated. Animals were killed by cervical dislocation 4 h after the paw challenge, and the depletion of mast cell population was estimated in groups of treated animals by counting the number of mast cells in the peritoneal cavity exudate, using toluidine blue.

Histopathological study

Animals were killed by cervical dislocation 4 h after the paw challenge. The paws were excised and the footpads fixed in 10% formalin solution. Paraffin blocks were prepared using conventional techniques and the histological sections stained with haematoxylin and cosin for light microscopic analysis.

Drugs and reagents

The following drugs and reagents were used: chicken OVA, dimethyl sulfoxide, compound 48/80, cromolyn, ketotifen, cyproheptadine, diphenrydramine, pentoxyfilline, indomethacin, nordihydroguaiaretic acid, NDGA (Sigma, St Louis, MO, USA), MK-886 (L-663,536(3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2 dimethylpropionic acid) (Calbiochem, La Jolla, CA, USA), tienotriazolobenzodiazepinic compound (WEB 2086; Institute Pasteur, Paris, France), methysergide (maleate; Sandoz/Novartis, Cambridge, MA, USA), meclizine (Pfizer, São Paulo, SP, Brazil), thalidomide (CEME, Brasília, Brazil), chlorpromazine (Rhodia Farma, São Paulo, SP, Brazil), dexamethasone (Decadron; Merck Sharp & Dohme, São Paulo, SP,
Brazil), and colloidal aluminum hydroxide (Sanofi, Rio de Janeiro, RJ, Brazil).

Statistical analysis

The results are expressed as means ± standard error of the mean (SEM). Statistical evaluation was undertaken by analysis of variance (ANOVA) following Student’s paired t-test. Statistical differences were considered significant at $p < 0.05$.

Results

Dose dependence and time-course of paw oedema induced by OVA

The intraplantar injection of 10 µg/paw of OVA induced a sustained oedema in rats immunized with OVA at doses up to 200 µg/rat (Fig. 1). At the lowest doses (10 µg/rat) of sensitization, the oedema had a rapid onset, peaking 1 h after injection. For the dose used in this study (30 µg/rat to sensitization and
10 μg/paw to challenge), the oedema reached a peak at about 2 h after the challenge, followed by a gradual decrease thereafter, and at 24 h post injection it was almost absent. Furthermore, the oedema increased up to 21 days after sensitization (Fig. 2), while the highest doses (200 μg/rat) induced a long-lasting oedema, which increased up to 28 days after sensitization (data not shown). Non-sensitized or control groups sham-sensitized with Al(OH)$_3$ were not affected by intraplantar OVA.

Response of OVA-induced oedema to standard antagonists

Pretreatment of the animals with dexamethasone (0.5mg/kg) was effective in inhibiting allergicoedema. Using non-steroidal anti-inflammatory agents, indomethacin (2mg/kg), MK-886 (10mg/kg), NDGA (60mg/kg) and WEB 2086 (5mg/kg), we found that cyclooxygenase and lypoxygenase products do not participate in the reaction. The combination of indomethacin (2mg/kg) + MK-886 (10mg/kg) + ketotifen (10mg/kg) also failed to reduce the oedema induced by OVA (Table 1). All used doses have previously been shown to inhibit the corresponding pathways of arachidonic acid metabolism in rats.$^{11}$

The role of mast cell and endogenous amines on OVA-induced oedema

Vasoactive amines appear to be involved because anti-histamine and anti-serotonin agents reduced the oedema. A significant inhibition of OVA-induced oedema was observed with methysergide (anti-serotonin), diphenhydramine (anti-histamine) and cyproheptadine (anti-histamine and anti-serotonin) (Table 2, and Figs 3 and 4). Inhibition induced by diphenhydramine was dose dependent for all doses (25, 50, 75 and 100mg/kg, s.c.). The highest dose (100mg/kg) almost completely blocked oedema formation by

---

### Table 1. Failure of standard agents to inhibit paw oedema induced by OVA in sensitized rats

| Treatment | Dose (mg/kg) | Time |
|-----------|-------------|------|
|           |             | 1    | 2    | 3    | 4    |
| PBS (n = 6) |             | 0.70 ± 0.10 | 0.65 ± 0.06 | 0.60 ± 0.04 | 0.53 ± 0.03 |
| Indomethacin (n = 6) (cyclo-oxygenase inhibitor) | 2 | 0.73 ± 0.05 | 0.63 ± 0.03 | 0.6 ± 0.05 | 0.52 ± 0.05 |
| MK-886 (n = 5) (5-lipoxygenase inhibitor) | 10 | 0.54 ± 0.09 | 0.57 ± 0.10 | 0.48 ± 0.08 | 0.39 ± 0.09 |
| NDGA (n = 5) (cyclooxygenase and lypoxygenase inhibitor) | 60 | 0.72 ± 0.06 | 0.80 ± 0.08 | 0.72 ± 0.07 | 0.60 ± 0.06 |
| WEB 2086 (n = 6) (PAF antagonist) | 5 | 0.58 ± 0.02 | 0.60 ± 0.04 | 0.54 ± 0.03 | 0.46 ± 0.03 |
| Ketotifen (n = 6) (mast cell stabilizer) | 10 | 0.62 ± 0.03 | 0.55 ± 0.02 | 0.56 ± 0.03 | 0.50 ± 0.03 |
| Combination (n = 6)$^b$ | – | 0.56 ± 0.06 | 0.59 ± 0.03 | 0.58 ± 0.02 | 0.52 ± 0.02 |

Data expressed as the mean ± SEM of the increase in paw volume (ml).

$^a$Ovalbumin (10μg/100μl) was intraplantar injected and oedema was measured after 1, 2, 3 and 4h. Number of animals/group in parentheses.

$^b$Combination = indomethacin + MK 886 + ketotifen at indicated doses.

### Table 2. Effect of anti-histamine and anti-allergic drugs on the time course of paw oedema induced by OVA in sensitized rats

| Treatment | Dose (mg/kg) | Time |
|-----------|-------------|------|
|           |             | 1    | 2h   | 3h   | 4h   |
| PBS (n = 6) |             | 0.70 ± 0.10$^c$ | 0.65 ± 0.06 | 0.60 ± 0.04 | 0.53 ± 0.03 |
| Meclizine (n = 5) (H$_1$ anti-histamine) | 30 | 0.64 ± 0.06 | 0.52 ± 0.04 | 0.55 ± 0.05 | 0.47 ± 0.07 |
| Methysergide (n = 5) (5-HT$_2$ blocker) | 5 | 0.21 ± 0.04$^*$ | 0.36 ± 0.06$^*$ | 0.25 ± 0.06$^*$ | 0.26 ± 0.07$^*$ |
| Ketotifen (n = 6) (mast cell stabilizer) | 10 | 0.62 ± 0.03 | 0.55 ± 0.02 | 0.56 ± 0.03 | 0.50 ± 0.03 |
| Cromolyn (n = 6) (mast cell stabilizer) | 5 | 0.73 ± 0.06 | 0.87 ± 0.06$^*$ | 0.72 ± 0.04 | 0.61 ± 0.05 |
| Compound 48/80 (n = 6) (depletor of mast cell) | 0.85 ± 0.05 | 0.92 ± 0.03$^*$ | 0.84 ± 0.05$^*$ | 0.68 ± 0.03$^*$ |
| Dexamethasone (n = 5) (glucocorticoid) | 0.5 | 0.29 ± 0.02$^*$ | 0.41 ± 0.02$^*$ | 0.38 ± 0.03$^*$ | 0.23 ± 0.02$^*$ |

Data expressed as the mean ± SEM of the increase in paw volume (ml).

$^a$Ovalbumin (10μg/100μl) was intraplantar injected and oedema was measured after 1, 2, 3 and 4h. Number of animals/group in parentheses.

$^b$Combination = indomethacin + MK 886 + ketotifen at indicated doses.

$^c$Combination = indomethacin + MK 886 + ketotifen at indicated doses.

$^d$p < 0.05 (ANOVA, Student’s t-test), compared with the PBS group.
OVA (Fig. 3). In contrast, meclizine (classical anti-H\textsubscript{1}) failed to inhibit immune oedema.

Effect of mast cell stabilizer agents and compound 48/80 on oedema induced by OVA

Pretreatment of animals with cromolyn (disodium cromoglycate) slightly influenced the oedema induced by OVA even at doses up to 20 mg/kg. Cromolyn induced stimulation at a lower dose of 5 mg/kg that was only detectable at 2 h (Table 2). On the contrary, ketotifen (10 mg/kg) was not effective (Table 2). In addition, the depletion of mast cells by systemic treatment with compound 48/80 slightly, but significantly, increased the oedematogenic response (Table 2).
Response of immune oedema to anti-tumour necrosis factor drugs

In the present work, the effect of drugs like thalidomide, inhibitors of tumour necrosis factor (TNF) release, and pentoxyfilline, inhibitor of TNF and interleukin-1 synthesis, was investigated. Table 3 shows that thalidomide (90 mg/kg) and pentoxyfilline (90 mg/kg) did not affect the oedema induced by OVA in rats. However, a significant inhibition by chlorpromazine (inhibitor of TNF synthesis) was noted for all doses (Table 4).

Histological study of the rat paws after antigenic challenge

An intense infiltration of neutrophils and eosinophils was observed in the hypodermis of the paws injected with OVA.

Discussion

Our results clearly demonstrate that immune oedema do not appear to be dependent on arachidonic acid metabolism, since the cyclooxygenase inhibitor (indomethacin), the 5-lipoxygenase inhibitor (MK-886) and the PAF antagonist (WEB 2086), or the combination of indomethacin + MK-886 + ketotifen, exerted no significant inhibition on this oedema, when tested at doses that have been commonly used. On the contrary, dexamethasone had a marked inhibitory effect on the oedema.

The inhibitory effect of dexamethasone on oedema of the rat paw induced by OVA is probably not due to interference with eicosanoid formation, since drugs that block cyclooxygenase and lipoxygenase were ineffective. Several effects of glucocorticoids may be explained by their capacity to block the release of chemotactic mediators as the metabolites of arachidonic acid and/or cytokines with inflammatory properties. The inhibitory effect of dexamethasone on allergic oedema may result, at least in part, from the inhibition of the release of inflammatory cytokines.

The role of vasoactive amines was also investigated in this experiment. The effect of two chemically

Table 3. Effect of anti-TNF drugs on the time-course of paw oedema induced by OVA in sensitized rats

| Treatment         | Dose (mg/kg) | 1      | 2      | 3      | 4      |
|-------------------|-------------|--------|--------|--------|--------|
| PBS (n = 6)       | 0.68 ± 0.08 | 0.69 ± 0.07 | 0.59 ± 0.04 | 0.52 ± 0.03 |
| Thalidomide (n = 6) | 90         | 0.65 ± 0.07 | 0.71 ± 0.07 | 0.66 ± 0.06 | 0.63 ± 0.06 |
| Pentoxifylline (n = 6) | 90       | 0.64 ± 0.03 | 0.64 ± 0.04 | 0.50 ± 0.04 | 0.48 ± 0.05 |

Data expressed as the mean ± SEM of the increase in paw volume (ml).

Table 4. Effect of chlorpromazine (anti-TNF/5-HT2) on the time course of paw oedema induced by OVA in sensitized rats

| Treatment         | Dose (mg/kg) | 1      | 2      | 3      | 4      |
|-------------------|-------------|--------|--------|--------|--------|
| PBS (n = 15)      | 0.75 ± 0.05 | 0.73 ± 0.04 | 0.66 ± 0.04 | 0.56 ± 0.03 |
| Chlorpromazine (n = 6) | 1       | 0.50 ± 0.06* | 0.55 ± 0.05* | 0.46 ± 0.05* | 0.42 ± 0.05* |
| Chlorpromazine (n = 6) | 3       | 0.55 ± 0.07* | 0.58 ± 0.08* | 0.51 ± 0.07* | 0.4 ± 0.06* |
| Chlorpromazine (n = 6) | 9       | 0.26 ± 0.05* | 0.29 ± 0.02* | 0.28 ± 0.05* | 0.23 ± 0.04* |
| Chlorpromazine (n = 6) | 18      | 0.29 ± 0.04* | 0.34 ± 0.4* | 0.29 ± 0.04* | 0.31 ± 0.03* |

Data expressed as the mean ± SEM of the increase in paw volume (ml).

*Ovalbumin (10 μg/100 μl) was intraplantar injected and oedema was measured after 1, 2, 3 and 4 h. Number of animals/group in parentheses.

p < 0.05 (ANOVA, Student’s t-test), compared with the PBS group.
distinct hydrogen antagonists, meclizine and
diphenhydramine, was tested. It was found that only
diphenhydramine caused reduction in the immune
oedema in a dose-dependent manner. In addition,
methysergide (serotonin antagonist) inhibited OVA-
induced oedema with optimal effect at a dose of
5 mg/kg, as well as cyproheptadine (histamine and
serotonin antagonist), suggesting the involvement of
endogenous amines, probably serotonin.

Although diphenhydramine has a well-established
H₁ anti-histamine action, its anti-oedematogenic effect
in the sensitized rat may not necessarily be associated
with this property, since meclizine (classical anti-H₁)
failed to block the oedema. Thus, histamine seems not
to be involved. Meclizine’s lack of effect is not
surprising, since histamine is not an important
mediator of vascular permeability in rats. Tromp
et al. investigated the role of mast cells and histamine
in leukocyte–endothelium interactions in four rat
strains: Brown Norway, Lewis, Sprague–Dawley and
Wistar. In Sprague–Dawley rats, the topical admin-
istration of histamine (10⁻⁴ M) resulted in a significant
increase in the level of leukocyte rolling and a
decrease in the rolling velocity compared with the
time control. Histamine induced leukocyte adhesion
only in the Brown Norway strain.

Additionally, studies have apparently divided the
anti-histamine drugs into two classes: those com-
pounds that release histamine and serotonin from
isolated rat peritoneal mast cells, and those that
inhibit the release of these mediators by compound
48/80. Possible different effects of the anti-hista-
mine drugs have also been investigated. Diphenhy-
dramine appears to be effective in inhibiting ser-
otonin uptake. Maling et al. showed that
diphenhydramine inhibits oedema induced by ser-
otonin in rats. Thus, we speculate a possible effect of
this drug on the depletion of serotonin.

Serotonin is a naturally occurring amine with major
effects on a variety of bodily functions. Important
studies concerning serotonin have focused on vas-
cular and inflammatory responses. Owen suggested
that administering serotonin to the plantar surface of
the rat hind paw caused oedema with striking
extravasation of albumin. Serotonin was also reported
to induce plasma extravasation as a result of oedema
formation in other models. Honrubia et al. showed
that cyproheptadine inhibits oedema related to these
molecular features, which make feasible a common
disposition to interact with all three 5-HT₂ subtypes.
In addition, cyproheptadine is a drug that shows high affinity for
serotonin type 2 (5-HT₂) receptors. Honrubia et al.
showed that the activity of cyproheptadine
derivatives at 5-HT₂ receptors is related to these
molecular features, which make feasible a common
disposition to interact with all three 5-HT₂ subtypes.
Since methysergide, primarily a serotonin type 2
(5-HT₂) antagonist, and cyproheptadine, anti-
H₁/5-HT₂, are active inhibitors of oedema, we
suggest that serotonin may be an important mediator
in the formation of oedema induced by OVA in
sensitized rats, being the binding site of the receptor
5-HT₂.

On the contrary, the mast cell stabilizer com-
pounds, cromolyn and ketotifen, had a slight or
no effect in this oedema. Cromolyn (disodium
cromoglycate) pretreatment only slightly influ-
enced the inflammatory response. Results showed that
cromolyn at lower doses (5 mg/kg) slightly and
temporarily (2 h) increased the oedema, whereas at
higher doses (20 mg/kg) it slightly reduced the
oedema only in young rats (about 130 g body
weight) (data not shown). The action of cromolyn
on inflammation remains uncertain. This drug
inhibits mast cell degranulation and has a direct
effect on inflammatory cells. Shida suggests that
cromolyn probably, non-specifically, targets the sur-
face of relevant cells including mast cells and
cosinophils. In addition, some studies suggest that
cromolyn diminishes cell activation. In this
study, the stimulatory effect at lower doses sug-
gests that the involvement of mast cells may be in
the inhibition of this oedema, while the inhibitory
effect at higher doses in young rats may be due to
a direct effect on immune-inflammatory immature
cells, or due to another mechanism of unknown
basis.

Compound 48/80 is known as a potent inducer of
degranulation and of the release, from connective
tissue-type mast cells, of histamine and other chem-
ical mediators, which are responsible for anaphylactic
symptoms. In this work, OVA-induced oedema was
given potential by subchronic treatment by the mast
cell degranulator compound 48/80. Thus, the involve-
ment of mast cells may occur in the inhibition of this
oedema.

Histological analysis of the hind-paw 4 h after the
paw challenge showed an intense infiltration of
neutrophils and eosinophils in the hypodermis of the
paws injected with OVA (data not shown). Consider-
ing that in the OVA-injected paw there is a migration of
neutrophils and eosinophils to the extravascular
tissues, we attributed the contribution of leukocyte
migration to the development of allergic oedema. In
addition, TNFα induces neutrophil migration in immune
inflammation. Thus, we investigated the pos-
sibility that TNFα could be responsible for the
neutrophils’ chemotactic activity. Anti-TNF agents
were ineffective against OVA-induced oedema. Pen-
toxifylline is a methylxanthine-derivative drug that
has been used for more than 20 years in the treatment
of peripheral vascular disease. Pentoxifylline is also a
potent inhibitor of TNFα secretion, both in vitro and
in vivo, and has demonstrated its efficacy in the
treatment of certain animal and human inflammatory
diseases. Furthermore, thalidomide exerts its inhibi-
tory action on TNFα by enhancing mRNA degrada-
tion. Our data suggest that the factor responsible for
inducing cell migration is different from TNFα, since
thalidomide and pentoxifylline did not inhibit the immune oedema.

Additionally, in this study a wide range of doses were used for chlorpromazine (inhibitor of TNFα synthesis and implicated in several others functions), due to exploring this new literature data in this specific model. A significant inhibition by this drug was noted at all doses in this study. At higher doses (18 mg/kg), chlorpromazine produced a highly significant reduction in the oedemogenic effect. Chlorpromazine, a phenothiazine derivative, possesses anti-inflammatory properties, inhibiting TNFα synthesis and bone resorption. Also, the anti-serotonin effects of chlorpromazine at the 5HT2 site have been well characterized. Trichard et al. suggest a high level of 5-HT2A blockage with high doses of chlorpromazine. Its anti-histamine properties, however, are less well known.

In conclusion, the present work shows that the fact that indomethacin, MK-886, NDGA and WEB 2086 have not been able to inhibit allergic oedema, indicating that the action of dexamethasone seems not to be via phospholipase A2, but possibly due to the synthesis and/or activity inhibition of pro-inflammatory cytokines or via the inhibition of expression or function of adhesion molecules. Our results suggest that anaphylactic rat paw oedema is independent of histamine action. The paw oedema inhibition by diphenhydramine, but not by meclizine (classical anti-H1), may suggest a different mechanism, which is independent from the anti-histamine effect. These data suggest a role for serotonin in the rat oedema induced by OVA, since methysgeride and cyproheptadine were active inhibitors of this oedema. Thus, we speculate that serotonin is largely responsible for immunological oedema in rats, via 5-HT2 receptors, besides a possible involvement of other mediators, probably cytokines.

Acknowledgements. Research supported by FUNCAP.

References

1. Guo Y, Mochizuki T, Morii, E., Kitamura Y, Macymaya K. Role of mast cell histamine in the formation of rat paw edema: a microdialysis study. Eur J Pharmacol 1997; 351: 237–243.
2. Casey JB, Tokuda S. A comparative study of the mechanism of passive cutaneous anaphylaxis induced by mouse IgG, rabbit Fab2α antibodies. Int Arch Allergy Immunol 1973; 44: 735.
3. Kaneta S, Kanbara H, Fujihira E, Mitsuya M. Mouse IgE-mediated paw anaphylaxis in mice and rats. Int Arch Allergy Appl Immunol 1980; 60: 351.
4. Amorim CZ, Cordeiro RS, Vargaftig BB. Interference of antihistamines and anti-allergic drugs with antigen-induced paw edema in boosted and unboosted mice. Eur J Pharmacol 1992; 216: 429–434.
5. Bissonnette EY. Histamine inhibits tumour necrosis factor alpha release by mast cells through H2 and H5 receptors. Am J Respir Cell Mol Biol 1996; 14: 620–629.
6. Kim DY, Camilleri M. Serotonin: a mediator of the brain–gut connection. Eur J Pharmacol 1997; 351: 237–243.
7. Perdue MH, Chung MG, Gall DG. Effect of intestinal anaphylaxis on gut function in the rat. Gastroenterology 1984; 86: 591–597.
8. Scott RB, Tan DTM, Miambamba P, Sharkey KA. Anaphylaxis-induced alterations in intestinal motility: role of extrinsic neural pathways. Am J Physiol 1998; 275: G812–G821.
9. Lesser ML, Brown KI, Helson L. Statistical methods for measuring and comparing treatment efficacy application to nude mice experimentation. Eur J Cell Biol 1980; 41: 126–137.
10. Landucci ECT, Fantone J, et al. Inhibition of carrageenin-induced rat paw oedema by crotapotin, a polypeptide complexed with phospholipase A2. Br J Pharmacol 1995; 114: 578–583.
11. Rocha MFG, Maia MET, Bezerra LRPS, Ilyes B, Guerrero RL, Ribeiro RA, Lima AAM. Chlorpromazine inhibits the release of neuropeptide chomatostatic factors from rat peritoneal macrophages: role of interleukin-1β, tumour necrosis factor alpha, and leukotrienes. Infect Immun 1997; 65: 2740–2746.
12. Maling HM, Webster ME, Williams MA, Saul W, Anderson WA. Inflammation induced by histamine, serotonin, bradykinin and compound 48/80 in the rat: antagonists and mechanisms of action. J Pharmacol Exp Ther 1974; 191: 300–310.
13. Di Paola M, Giroud JP, Willoughby DA. Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol 1971; 104: 15–29.
14. Moreira AL, Sampaio EP, Zainuddin A, Frinidt P, Smith KA, Kaplan GH. Thromboxane exerts its inhibitory action on tumour necrosis factor alpha by enhancing mRNA degradation. J Exp Med 1995: 177: 1675–1680.
15. Endres S, Fulle HJ, Sinha B, Stoll D, Dinarello CA, Geracz R, Weber PC. Cyclic nucleotides differentially regulate the synthesis of tumour necrosis factor-alpha and interleukin-1 beta by human mononuclear cells. Immunology 1991; 72: 56–60.
16. Pollice PE, Rosier RN, Looney RJ, Puzas JE, Schwarz EM, O’Keefe RJ. Oral pentoxifylline inhibits release of tumour necrosis factor alpha from humuer peripheral blood mononcytes: a potential treatment for aseptic loosening of total joint components. J Bone Joint Surg Am 2001; 83A: 1057–1061.
17. Dinarello CA. The proinflammatory cytokines interleukin-1 and tumour necrosis factor and treatment of the septic shock syndrome. J Infect Dis 1991; 165: 1177–1814.
18. Da Motta JI, Cunha FQ, Vargaftig BB, Ferreira SH. Drug modulation of antigen-induced paw oedema in guinea-pigs: effects of lipopolysaccharide, tumour necrosis factor and leucocyte depletion. Br J Pharmacol 1994; 112: 111–116.
19. Kann S, Horváthová M, Gazdik F. Expression of adhesion molecules and effect of disodium cromoglycate treatment in asthmatics. Physiol Res 1998; 47: 439–443.
20. Xamaki K, Thottachary H, Xie Y, Lindbom L, Hedqvist P, Raun J. Characteristics of histamine-induced leucocyte rolling in the undisturbed microcirculation of the rat mesentery. Br J Pharmacol 1998; 123: 390–399.
21. Wilhelm DL. The modulation of increased vascular permeability in inflammation. Pharmacol Rev 1962: 14: 291–298.
22. Tropp SC, Talgedler GJ, Slaaf DW, et al. The role of mast cells and histamine in leucocyte–endothelium interactions in four rat strains. Pflugers Arch 1998: 436: 255–261.
23. Vela SY, Dorsch C, Rodin H, Cadet JL. Effects of antitamines on 5,4-methylenedioxymethylamphetamine-induced depletion of serotonin in rats. Synapse 1999; 33: 207–217.
24. Lee HZ, Wu CH. Serotonin-induced protein kinase C activation in cultured rat heart endothelial cells. Eur J Pharmacol 2000; 403: 195–202.
25. Owen DAA. Vascular changes during acute inflammation responses in rat hindpaws. In: Willoughby DA, Giroud JP, Velo GP, eds Perspectives in Inflammation. London: MTP, 1977: 491–495.
26. Pearce P, Xie GX, Peroukta SJ, Green PG, Levine JD. 5-Hydroxytryptamine-induced synovial plasma extravasation is mediated via 5-hydroxytryptamine 2A receptors on sympathetic efffrent terminals. J Pharmacol Exp Ther 1995; 275: 502–508.
27. Wang JP, Chen YH, Kuo SC. Inhibition of hind paw edema and cutaneous vascular plasma extravasation by 2-chloro-5-methoxy-carbonylpropiono-mido-1,4-naphthoquinone (PPDh) in mice. Naunyn-Schmiedeberg’s Arch Pharmacol 1995; 354: 779–784.
28. Horrubia MA, Rodriguez J, Dominguez R, Lozoya E, Manuel E, Seijas JA, Villeneuve MC, Calleja J, Castel MI, Maynayi S, Sany F, Loza ML. Synthesis, affinity at 5-HT2A, 5-HT2B and 5-HT2C serotonin receptors and structure-activity relationships of a series of cyproheptadine analogues. Chem Pharm Bull 1997; 45: 842–848.
29. Liston H, Bennett L, Usher B, Nappi J. The association of the combination of sumatriptan and methysergide infarction in a premenopausal woman. Arch Intern Med 1999; 159: 511–513.
30. Wolf C, Schunack W. Synthesis and pharmacology of combined histamine H1/H2-receptor antagonists containing diphenhydramine and cyproheptadine derivatives. Arch Pharm 1990; 329: 842–848.
31. Shida T. A comparison of the pharmacological actions between DSCG (disodium cromoglycate) and BDP (beclomethasone dipropionate) in the treatment of bronchial asthma. Nippon Rinsho 1996; 54: 301–306.
32. Busino L, Cantani A. Food allergy in children: diagnosis and treatment with sodium cromoglycate. Allergol Immunopathol (Madr) 1990; 18: 339–348.
33. Canetti C, Silva JS, Ferreira SH, Camilo FQ. Tumour necrosis factor-alpha.
and leukotriene B(2) mediate the neutrophil migration in immune inflammation. *Br J Pharmacol* 2001; 134: 1619–1628.

34. De Lima V, Bezerra MM, de Menezes Alencar VB, Vidal FD, da Rocha FA, de Castro Brito GA, de Albuquerque Ribeiro R. Effects of chlorpromazine on alveolar bone loss in experimental periodontal disease in rats. *Eur J Oral Sci* 2000; 108: 125–129.

35. Nishima K, Yoshino T, Yui K, Katoh S. Potent serotonin (5-HT)(2A) receptor antagonists completely prevent the development of hyperthermia in a rat animal model of the 5-HT syndrome. *Brain Res* 2001; 890: 23–31.

36. Trichard C, Pailhère-Martinot ML, Attar-Levy D, Recassens C, Monnet F, Martinot JL. Binding of antipsychotic drugs to cortical 5-HT(2A) receptors: a PET study of chlorpromazine, clozapine, and amisulpride in schizophrenic patients. *Am J Psychiatry* 1998; 155: 505–510.

Received 2 January 2002
Accepted 12 March 2002