Modulation of histamine release by fatty acids. A new *in vitro* model investigating adverse drug reactions in various species

Modulation de l'histaminolibération par les acides gras

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ABSTRACT : Histamine release caused by drugs and/or their solvents is a well known phenomenon. In this study, both *in vivo* (anaesthetized and conscious dogs) and *in vitro* (isolated rat peritoneal, human and guinea-pig lung mast cells) models were used. Cremophor E1 and six derivatives of 12-hydroxystearic acid were compared for their histamine releasing abilities. Although the three types of isolated mast cells responded similarly, histamine release being observed with DH (the diester of 12-hydroxystearic acid with polyethylene glycol), TN (12-hydroxystearic acid polymerized with ethylene oxide) and ME (the monoester of 12-hydroxystearic acid esterified with polyethylene glycol), the anaesthetized dog exhibited elevated blood histamine levels and clinical symptoms after administration of all the solubilizing agents, except TN and ME. The reasons for this discrepancy are not known. The addition of the drugs (Althesin or propanidid) to their solubilizing agents caused histamine release, which was not observed with the solubilizing agent alone. This is the first demonstration of an *in vitro* model, which copies the clinical situation, *i.e.* solvent does nothing but the solvent plus drug combination causes histamine release and hence adverse reactions.

RÉSUMÉ : La libération d'histamine, due à des produits anesthésiques et/ou à leurs solvants, est un phénomène bien connu. Deux modèles ont été utilisés au cours de cette étude : l'un *in vivo* (chez le chien anesthésié ou éveillé), l'autre *in vitro* (sur des mastocytes isolés de péritoine de rat, de poumon humain ou de cobaye). Les capacités histaminolibératrices du crémophor E1 et de six dérivés de l'acide 12-hydroxystéarique ont été comparées. Quoique les trois types de mastocytes isolés aient réagi de façon analogue, l'histaminolibération étant observée avec le DH (diester de l'acide 12-hydroxystéarique + polyéthylène glycol), le TN (acide 12-hydroxystéarique polymérisé avec de l'oxyde d'éthylène) et le ME (monoester de l'acide 12-hydroxystéarique estérifié avec du polyéthylène glycol), le chien anesthésié présente un taux d'histamine élevé et des symptômes cliniques après administration de tous les agents solvants, sauf le TN et le ME. Les raisons de cette différence ne sont pas connues. L'adjonction d'Alfatésine ou de propanidide aux agents solvants entraîne une histaminolibé-ration, qui n'était pas observée avec le solvant seul. Cette étude est le premier exemple d'un modèle *in vitro*, copiant la situation clinique : le solvant n'entraîne pas de réaction, mais l'adjonction de produit à ce dernier provoque une histaminolibération et donc des effets secondaires.

Adverse reactions to drugs used during anaesthesia and surgery are common. Although reported incidences vary considerably, using data obtained from 23 carefully controlled clinical trials, the following incidences were obtained : all reactions 20-30 %, systemic reactions 1-5 % and life-threatening reactions 0.1-0.5 % [8]. In many cases, these reactions appear to be caused not by the drug itself but by the combination of drug and solvent. For example, the hypnotic drug Althesin® was found to elevate plasma histamine levels and produce tachycardia and hypotension in volunteers but the solvent alone, cremophor E1, was without adverse effects [3,

9]. In studies using cats and dogs, however, this solvent alone produced elevation of whole blood and plasma histamine levels and alterations in haemodynamic parameters [10].

In order to reduce the number of adverse reactions, two approaches must be followed : 1) the investigation of the mechanisms of histamine release caused by the solvent or the solvent plus drug combination, 2) the development of less toxic solubilizing agents.

In this study, both *in vivo* and *in vitro* techniques have been used to investigate the histamine release caused by cremophor E1 and related compounds.

MATERIALS AND METHODS

Anaesthetized dog experiments

Anaesthetized mongrel dogs (both sexes) were prepared as previously described [13]. Briefly the dogs were anaesthetized with pentobarbitone, two catheters were inserted and 20 min allowed to elapse for stabilization. Two blood samples were removed before drug administration (to provide basal histamine levels) and then 2, 5, 10, 15 and 30 min after drug administration. The solubilizing agent was given as an intravenous bolus $(0.1 \; ml \cdot kg^{-1})$. Histamine was determined in whole blood using the combined fluorometric-fluoroenzymatic method [7].

Conscious dog experiments

Beagle dogs (12-18 months old, both sexes) with two indwelling catheters were used. The drug was administered $(200 \; mg \cdot kg^{-1})$ via a catheter in the jugular vein; blood sampling (via the cephalic vein catheter) was performed at $-5, -1, 3, 5,$ 10, 15 and 30 min after drug injection. Histamine was determined in plasma, using the combined fluorometric-fluoroenzymatic assay of LORENZ and coworkers [7].

Isolated mast cells

Mast cells were obtained from human lung [4], guinea-pig lung [2] and the peritoneal cavity of the rat [1] by previously described methods. Briefly the lung tissue was used as soon as possible after organ excision. The tissue was cut into pieces and washed thoroughly with Tyrode's-Hepes buffer; it was then chopped into $1 \; mm^2$ chunks using a McIllwain tissue chopper and finally enzymatically dispersed for 90 min at 37 °C using collagenase type 1 (Sigma) (0.05 % in Tyrode's-Hepes buffer containing bovine serum albumin 0.1 %). The tissue was then mechanically disrupted, filtered through gauze and the cells recovered by centrifugation. The cells were then washed twice with Tyrode's-Hepes and then used as described below.

Rat peritoneal mast cells were obtained from female Sprague-Dawley rats. These animals were anaesthetized with ether and then killed by cervical dislocation and exanguination. Tyrode's-Hepes buffer plus heparin was injected into the peritoneal cavity, the abdomen was massaged for 90 s and the cells then removed. These cells were then washed (twice) and recovered by centrifugation. The cell suspensions were then used as described below.

The cell suspensions (to a final volume of 1 ml) were prewarmed for 5 min at 37 °C. The drug was added and histamine release allowed to proceed for 10 min. The reactions were terminated by the addition of ice-cold buffer and the cells and supernatants separated by centrifugation.

Histamine was determined in both portions by the combined fluorometric-fluoroenzymatic assay [7]. The results are expressed as percentage histamine released, i.e. : % histamine release = histamine in supernatant/(histamine in supernatant + cells) × 100. The spontaneous histamine release, i.e. the histamine release in the absence of any stimulus (ca 5 %), has been subtracted from all results in this communication. Positive controls, included in each experimental run, used the calcium ionophore A 23187 $(10^{-6} \; M)$ and resulted in the release of ca 70 % of the total histamine.

Materials

Cremophor E1 and six derivatives of 12-hydroxystearic acid were the seven solubilizing agents tested in this study (generously provided by BASF, Ludwigshafen, FRG). The six derivatives of 12-hydroxystearic acid (12-HSA) were as follows : TN (12-HSA polymerized with ethylene oxide; degree of polymerization 15;

15 EO), MO (ethoxylated monoglyceride of 12-HSA; 46 EO), ET (ethoxylated triglyceride of 12-HSA; 48 EO), DH (diester of 12-HSA esterified with polyethylene glycol 600; PEG), ED (ethoxylated diester of 12-HSA with PEG; 10 EO) and ME (monoester of 12-HSA esterified with PEG).

Preparations of Althesin and its solubilizer were provided by Glaxo; Bayer supplied the samples of propanidid and its solubilizer; the calcium ionophore A 23187 was purchased from Boehringer.

RESULTS

Anaesthetized dog experiments

In this model, all the solubilizing agents tested, with the exception of TN and ME, released clinically significant amounts of histamine $(>10 \; ng \cdot ml^{-1}$ canine blood; data not shown). The careful examination of clinical symptoms and parameters such as redness, oedema, blood pressure changes, etc., confirmed these measurements.

Conscious dog experiments

TN $(200 \; mg \cdot kg^{-1})$ was administered to the conscious beagles on four separate occasions (table I). Histamine release was reduced on the second application, but higher on the third occasion than on the first. The fourth application resulted in either an identical release to the first or a markedly higher release.

Table I. — Repeated application of TN in conscious beagles

| | Maximum rise in plasma histamine content $(ng \cdot ml^{-1})$ | | | |
| | Dog 2801 | | Dog 4169 | |
	Basal	After drug	Basal	After drug
Day 1	3.0	96.3	2.4	26.2
Day 2	0.9	66.3	3.0	4.5
Day 5	4.4	144.3	0.9	39.3
Day 8	2.3	96.2	2.3	219.0

Isolated mast cell experiments

In contrast to the results obtained with anaesthetized dogs, only three solubilizing agents released histamine from the three mast cell preparations (human lung, guinea-pig lung and rat peritoneal mast cells). The three solubilizing agents which released histamine in a dose-dependent manner were TN, DH and ME. The results obtained with the human lung cells and the rat peritoneal mast cells are illustrated in figure 1, those obtained with guinea-pig lung cells being omitted for clarity. The maximal response to TN was ca 22 % (human lung), ca 38 % (guinea-pig lung) and ca 58 % (rat peritoneal mast cells). DH produced ca 50 % (rat peritoneal mast cells), ca 60 % (human lung) and ca
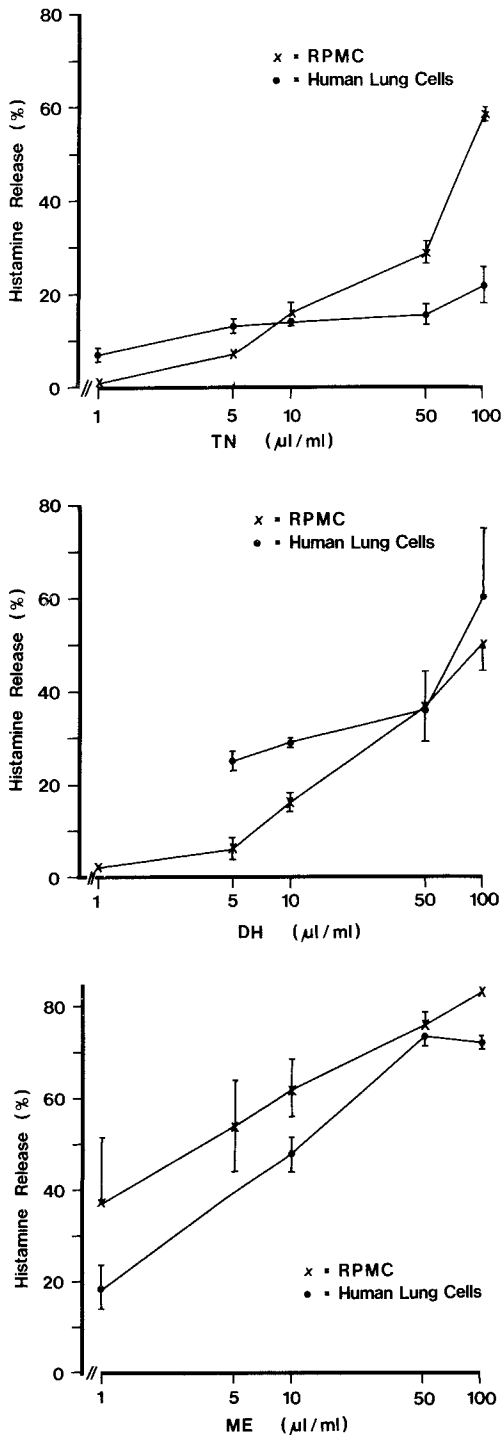
Fig. 1. — Histamine release induced by three solubilizing agents (TN, DH and ME) from rat peritoneal mast cells (RPMC) and human lung cells. All values are mean ±SEM, for n = 3-4 experiments.
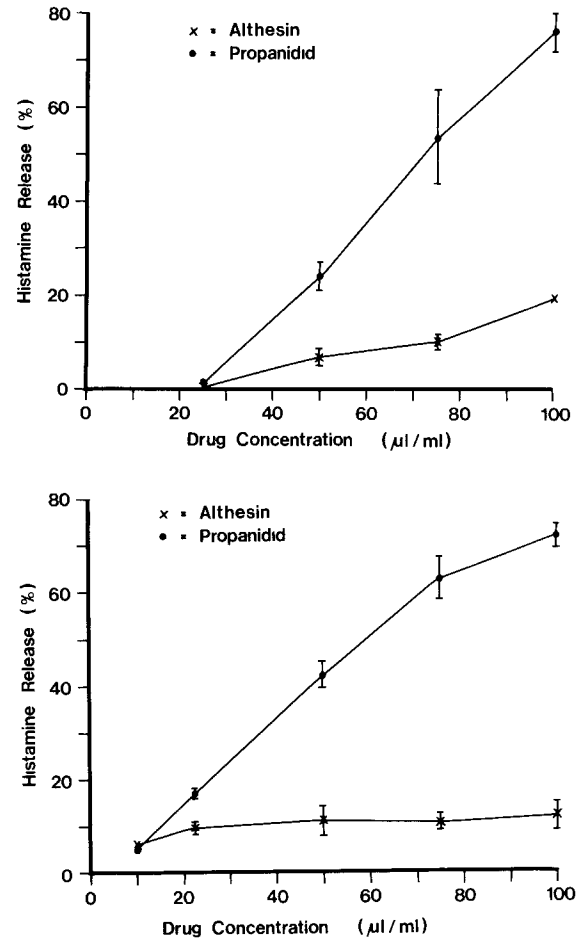

Fig. 2. — Histamine release induced by Althesin and propanidid from rat peritoneal mast cells (top panel) and guinea-pig lung cells (bottom panel). All values are mean ± SEM, for n = 3-4 experiments.

and guinea-pig lung cells (fig. 2). In both cases the solvent alone elicited no histamine release, but the drug plus solvent combination released histamine. In both cases propanidid was much more active.

DISCUSSION

There has been much work published on the histamine releasing ability of cremophor and its derivatives using in vivo models [11-13]. Cremophor E1, produced by the oxethylation of castor oil at elevated temperatures in an alkaline medium, is a mixture of hydrophilic and hydrophobic components [12]. In the anaesthetized dog model, the hydrophilic components did not release histamine. Of the hydrophobic components (composed of 80 % derivatives of ricinoleic acid, 5 % derivatives of oleic acid), only the derivatives of oleic acid released histamine [11]. Further experimentation indicated that the derivatives of 12-hydroxystearic acid were the least dangerous in this test system [13]. In the first part of the

65 % (guinea-pig lung). In all three cell types, ME produced near maximal histamine release (70-80 %).

The action of Althesin and propanidid and their solvents was examined using rat peritoneal mast cells

study here presented, cremophor E1 and six derivatives of 12-hydroxystearic acid were compared using two models : the anaesthetized dog and isolated mast cells. The results differed markedly in both systems. Only one compound (DH) was active in both test systems. Indeed the two substances exhibiting no activity in the anaesthetized dog model (TN, ME) were active in the isolated cell systems. It is interesting to note that the response to both ME and DH was similar in all three cell types examined; only the response to TN exhibited any major species difference. In previous studies investigating a wide range of histamine releasing agents, human and guinea-pig lung cells were much less reactive than the rat peritoneal mast cells [2, 4]. The solubilizing agents have been further examined in rat peritoneal mast cells and the results indicated that the response obtained occurs via a cytolytic mechanism [6]. However, although cremophor E1 and TN have identical tenside and micelle-forming properties (Lang, personnal communication), the responses obtained were very different, cremophor being inactive and TN active in the cellular systems.

The results obtained using conscious beagles are the first examining the effect of this solubilizing agent on non-anaesthetized dogs. The dose used in these experiments was approximately double that in the anaesthetized dog studies. Adverse reactions to cremophor E1 were previously thought to fall into two categories : the dog model where the animal reacts on first exposure and the pig model where the animal reacts on second exposure [5]. In these experiments, although the dogs reacted on first exposure and had a reduced reaction on second exposure, the third exposure (5 days after the first) elicited a much greater response.

The data presented in the final part of the result section demonstrates the first evidence for a model which resembles the clinical situation. Mast cells from two species (rat and guinea-pig) did not respond to the solvent alone but only to the combination of drug plus solvent. In both species, the drug propanidid elicited the most histamine release.

In 1984, both Althesin and propanidid were removed from the market in many European countries; it might therefore be thought that research such as presented in this paper is no longer clinically relevant. However, many drugs are solubilized with the aid of cremophor, including steroid hormones, antibiotics, immunosuppressants and vitamins. In many other preparations, e.g. cosmetics, small amounts of cremophor are present. The mechanisms of histamine release that are currently being investigated include :

— A direct disruptive action on the mast cell surface : probably true for DH, TN and ME but not for cremophor itself, since this solvent alone does not produce histamine release from isolated cells;

— an immunological mechanism involving e.g. complement activation : a possible mechanism, since WAT-

KINS [14] found an involvement of complement in 81 % of the adverse reactions to Althesin which he had investigated;

— a direct but selective action of the drug plus solvent combination on the mast cell surface : possible and under investigation.

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The influence of H_2 receptor antagonists on the plasma concentrations of midazolam and temazepam.
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Chez des volontaires sains, a été étudié l'effet d'une prémédication de 24 h avec 450 mg de ranitidine et 1 200 mg de cimétidine, en comparant avec du placebo sur les niveaux plasmatiques de deux benzodiazépines administrées oralement en une prise unique : 15 mg de midazolam (rapport d'extraction hépatique élevé) ou 20 mg de témazépam (rapport d'extraction hépatique bas). La concentration plasmatique du midazolam est significativement supérieure après ranitidine ou cimétidine par comparaison avec le placebo; la concentration plasmatique du témazépam n'est nullement affectée par la prémédication. La mesure des paramètres pharmacocinétiques montre que ranitidine et cimétidine augmentent d'environ 70 % la biodisponibilité du midazolam, alors que celle du témazépam n'est pas modifiée.

Ces résultats suggèrent que ranitidine et cimétidine affectent le fonctionnement hépatique. Il était déjà connu que ces deux antagonistes des récepteurs H_2 réduisaient le débit sanguin hépatique et que la cimétidine interférait avec le métabolisme hépatique des substances par l'intermédiaire de sa structure imidazole réagissant avec le cytochrome P_{450}. Or la ranitidine, ne possédant pas une telle structure, n'aurait pas d'effet sur le système enzymatique microsomal. Ces notions sont actuellement controversées et il semblerait que la ranitidine puisse se lier avec ce système enzymatique, expliquant l'augmentation des concentrations plasmatiques et de la demi-vie d'élimination des substances à métabolisme hépatique.

La prémédication anesthésique à visée antihistaminique par un anti-H_2 risque donc de modifier la durée d'action des substances à métabolisme hépatique utilisées pour l'anesthésie.

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