GENERAL ANAESTHETICS INDUCE ONLY HISTAMINE RELEASE SELECTIVELY FROM HUMAN MAST CELLS

C. STELLATO, V. CASOLARO, A. CICCARELLI, P. MASTRONARDI, B. MAZZARELLA AND G. MARONE

SUMMARY

We have examined the in vitro effects of increasing concentrations of propofol (5-70 μg ml⁻¹), ketamine (10⁻⁶–10⁻³ mol litre⁻¹) and thiopentone (10⁻⁵–8 × 10⁻⁴ mol litre⁻¹) on the release of preformed histamine and de novo synthesized mediators (peptide leukotriene C₄ (LTC₄) or prostaglandin D₂ (PGD₂)) from human basophils and mast cells isolated from lung parenchyma and skin tissue and from heart fragments. Propofol, ketamine and thiopentone failed to induce the release of histamine and de novo synthesis of LTC₄ from basophils. Propofol induced histamine release from lung (mean 8.6 (SEM 1.6)%) and skin mast cells (3.8 (1.5)%), but not from heart mast cells. Ketamine caused release of histamine from lung (6.2 (0.9)%) and skin mast cells (2.5 (1.5)%). Thiopentone caused a small amount of histamine release from lung mast cells (3.1 (1.2)%). Propofol, ketamine and thiopentone did not induce de novo synthesis of PGD₂ and LTC₄ from lung and skin mast cells. These results demonstrate that general anaesthetics induce only histamine release selectively from human mast cells.

KEY WORDS

Anaesthetics, intravenous: propofol, ketamine, thiopentone. Allergy: anaphylaxis, histamine.

The mechanisms of adverse reactions to general anaesthetics are complex [10]. However, whatever the mechanism(s), the resulting clinical manifestations are attributed commonly to histamine release by human mast cells and basophils [6, 7, 11]. This is supported by the findings that general anaesthetics cause histamine release in vitro [12] and in vivo [6, 7] and that administration of histamine reproduces most of the metabolic and haemodynamic effects observed during anaphylactic/anaphylactoid reactions [13-15].

An early commercial preparation of propofol was found to produce a high incidence of adverse reactions [16], presumably as a result of the histamine releasing capacity of the solubilizing agent, Cremophor EL [7, 17]. Propofol was therefore re-formulated, solubilized in a soya bean oil emulsion. However, the in vivo administration of propofol may produce skin flushing and a transient increase in plasma concentrations of histamine [17]. Despite these in vivo observations, the capacity of propofol to induce in vitro the release of histamine from human basophils and mast cells isolated from different anatomical sites has never been studied.

Techniques are now available to isolate large numbers of mast cells from human lung parenchyma [18], skin tissue [19] and human heart fragments [20]. These techniques have led to the demonstration that human basophils and mast cells isolated from different anatomical sites vary markedly as to their morphological, bio-
chemical and functional responses [18, 21-23]. Heterogeneity among these cells has been demonstrated with respect to the releasing activity of various stimuli and the release of different preformed histamine and de novo synthesized mediators (peptide leukotriene C₄ (LTC₄) and prostaglandin D₂ (PGD₂)) [19, 24].

Preliminary evidence suggests that human basophils and mast cells may also vary in their histamine releasing capacity when challenged with general anaesthetics. For example, thiopentone activates skin mast cells [12] but not human basophils. It should be emphasized that previous studies have focused on the histamine releasing capacity of anaesthetic drugs, but have not evaluated their possible effects on the de novo synthesis of chemical mediators.

We have investigated the ability of three i.v. general anaesthetic agents used commonly in clinical practice (propofol, ketamine and thiopentone) to induce the release of preformed and de novo synthesized mediators from human basophils and mast cells isolated from skin tissues, lung parenchyma and heart fragments.

MATERIALS AND METHODS

Reagents

The following reagents were used: Dextran 70 (Pharmacia Fine Chemicals, Uppsala, Sweden); piperazine - N,N'-bis (2-ethanesulfonic acid) (PIPES), elastase type I, hyaluronidase, chymopapain, collagenase, synthetic LTC₄ and PGD₂ (Sigma Chemical Co., St Louis, MO); Hank’s balanced salt solution (HBSS), fetal calf serum (FCS) (Gibco, Grand Island, NY); A23187, DNAase, pronase (Calbiochem, La Jolla, CA); RPMI 1640 with Hepes buffer 25 mmol litre⁻¹, Eagle’s minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland); [³H-LTC₄ (39.3 Ci mmol⁻¹) and [³H-PGD₂ (210 Ci mmol⁻¹) (New England Nuclear, Boston, MA). The following i.v. anaesthetic agents were used: propofol (Imperial Chemical Industries PLC, Macclesfield, U.K.), ketamine (Parke-Davis S.p.A., Milan, Italy) and thiopentone (Abbott S.p.A., Latina, Italy).

Histamine release from human basophils

After informed consent was obtained from all subjects, approximately 50 ml of blood was drawn into a final concentration of EDTA 0.008 mol litre⁻¹ and 1.1 % Dextran 70 [25]. The procedure for the isolation of peripheral blood basophils and the in vitro mediator release have been described in detail elsewhere [24, 25]. The cell-free supernatant was assayed for histamine with an automated fluorimetric technique [26]. The net percentage release was calculated by subtracting the histamine released spontaneously from the unstimulated aliquots (mean 2.8 (SEM 0.8) %) from the total histamine released from cell aliquots lysed with 2 % perchloric acid [24]. The difference between replicate histamine measurements was less than 10 %.

Purification of human skin mast cells

Skin was obtained from either mastectomies for breast cancer or elective cosmetic surgery procedures. General anaesthesia in these patients was performed using the following drugs: thiopentone, pancuronium, atropine, fentanyl, enflurane or isoflurane.

Tissue was placed immediately in Eagle’s MEM at 4 °C and used within 1 h. The skin was separated from the subcutaneous fat by blunt dissection and fragments were washed twice in calcium- and magnesium-free HBSS (CMF-HBSS) at 22 °C and incubated in a solution of CMF-HBSS containing collagenase 20 mg/g wet weight of tissue, hyaluronidase 5 mg/g wet weight of tissue and DNAase 1000 u ml⁻¹ for 3 h at 37 °C under constant stirring [19, 27]. The isolated cells were separated from the partially digested tissue fragments by filtration through Nytex cloth (150-µm pore size) and stored. The remaining tissue was digested a second time with a fresh batch of the enzymes for a further 2 h at 37 °C. The cells were separated from the tissue fragments by filtration through Nytex cloth, washed twice, combined with the cells from the first digestion, and washed again in CMF-HBSS. Yields obtained with this technique were 1–8 x 10⁶ mast cells/g wet weight of tissue, and purities varied between 1 % and 4 %.

Purification of human lung mast cells

Human lung tissue was obtained from patients undergoing thoracotomy and lung resection, mostly for lung cancer. General anaesthesia in these patients was performed using the following drugs: droperidol plus fentanyl and atropine (premedication); droperidol plus fentanyl, thiopentone, suxamethonium and pancuronium (anaesthesia). Lung parenchymal mast cells were...
isolated as described previously [18]. Yields obtained with this technique were 3–8 × 10^4 mast cells/g wet weight of tissue, and purities varied between 1% and 8%. Mast cells and basophils were stained with Alcian blue and counted in a Spiers–Levy eosinophil counter [18, 28].

**Histamine release from human skin and lung mast cells**

Aliquots of the cell suspension (0.4 ml) containing approximately 3 × 10^4 mast cells per tube were placed in Falcon 12 × 17 mm polyethylene tubes and warmed at 30 °C (skin mast cells) or 37 °C (lung mast cells); 0.2 ml of each releasing stimulus was added and incubation was continued at 30 °C (skin mast cells) or 37 °C (lung mast cells) for 30 min. Experiments with mast cells obtained from skin of adult donors were performed at 30 °C because adult skin mast cells release better at 30 °C than at 37 °C [19]. The net percentage release was calculated by subtracting the histamine released spontaneously from the unstimulated aliquots of skin (6.2 (0.7)% and lung mast cells (12.9 (1.6)% from the total histamine release from cell aliquots lysed with 2% perchloric acid [18, 24].

**Histamine release from human heart fragments**

Specimens of human right atrial appendages were obtained from patients undergoing cardiac surgery for valvular correction or substitution and aorto–coronary vein graft [20]. General anaesthesia in these patients was performed using the following drugs: thiopentone or diazepam, morphine or fentanyl, pancuronium, and nitrous oxide. None of the patients had taken dipyridamole, aspirin or any anti-inflammatory medication for at least 7 days before the operation. The procedure to evaluate the release of histamine from cardiac mast cells has been described in detail in previous publications [20, 23].

**Radioimmunoassay of LTC\(_4\) and PGD\(_2\)**

LTC\(_4\) and PGD\(_2\) concentrations were measured by radioimmunoassay as described previously [24, 25, 29]. The rabbit anti-LTC\(_4\) and anti-PGD\(_2\) antisera have been characterized and their cross-reactivity for heterologous ligands described elsewhere [29, 30].

**Statistical analysis**

Results were expressed as mean (SEM). Statistical analysis was performed by two-way non-parametric analysis of variance (Friedman test); multiple comparisons were performed by the extended Tukey test [31].

**RESULTS**

We evaluated the effects of increasing concentrations (5–70 μg ml\(^{-1}\)) of propofol [32] on histamine release from basophils and mast cells isolated from lung parenchyma and skin tissue. Propofol, in concentrations which included those reached during general anaesthesia [32], failed to induce histamine release from basophils obtained from 11 normal donors (fig. 1). We also determined the effects of propofol on the release of histamine from mast cells isolated from lung parenchyma obtained from nine donors. Propofol induced histamine release from lung mast cells in a concentration-dependent manner (P < 0.001). The maximum per cent histamine release caused by propofol varied between 3% and 18% (8.6 (1.6)% (P < 0.01 compared with spontaneous release). In three of nine skin mast cell preparations, propofol induced the release of more than 5% of the histamine content (P < 0.05). The greatest concentration of propofol used induced the release of 3.8 (1.5)% of skin mast cell histamine content (P < 0.05 compared with spontaneous release). It is noteworthy that the

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**Fig. 1.** Effect of various concentrations of propofol on histamine release from human basophils (○), lung parenchymal mast cells (HLMC) (□) and skin mast cells (HSMC) (△) (mean (SEM) obtained in 11 (basophils) and nine (HLMC and HSMC) experiments). *P < 0.05; **P < 0.01 compared with spontaneous release.
TABLE I. Effect of various concentrations of propofol (10–70 μg ml⁻¹) on histamine release (ng/g wet weight of tissue) from human heart mast cells (mean of duplicate samples). — = Measurement not made

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Buffer</th>
<th>Propofol (μg ml⁻¹)</th>
<th>Histamine released (ng g⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>1</td>
<td>74.0</td>
<td>75.0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>33.5</td>
<td>36.7</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>41.0</td>
<td>35.0</td>
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<tr>
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<td>5</td>
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<td>50.0</td>
<td>28.0</td>
</tr>
<tr>
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</tr>
<tr>
<td>7</td>
<td>26.0</td>
<td>14.0</td>
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</table>

Mean (SEM) 39.8 (7.0) 40.1 (8.4) 27.6 (0.8) 37.8 (5.0)

Histamine-releasing activity of propofol from lung and skin mast cells varied markedly among different donors. In seven experiments, increasing concentrations of propofol failed to induce the release of histamine from mast cells present in human heart (table I).

In a second series of experiments, we compared the effects of increasing concentrations (10⁻⁶–10⁻³ mol litre⁻¹) of ketamine, including those reached in vivo [32], on histamine release from basophils and lung or skin mast cells.

TABLE II. Effect of ketamine, anti-IgE and A23187 on the de novo synthesis of LTE₄ or PGD₂ from human basophils and lung (HLMC) and skin mast cells (HSMC). * Concentrations used: 0.3 μg ml⁻¹ in basophils, 1 μg ml⁻¹ in HLMC, 3 μg ml⁻¹ in HSMC. † Concentrations used: 0.2 μg ml⁻¹ in basophils, 1 μg ml⁻¹ in HLMC and HSMC. — = Not detectable

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Ketamine (mol litre⁻¹)</th>
<th>LTE₄ (ng/10⁶ basophils)</th>
<th>LTE₄ (ng/10⁶ HLMC)</th>
<th>PGD₂ (ng/10⁶ HSMC)</th>
</tr>
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<tr>
<td>Experiment 1</td>
<td>3 x 10⁻⁴</td>
<td>—</td>
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<td>—</td>
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<td>10⁻³</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Anti-IgE*</td>
<td>38.0</td>
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</tr>
<tr>
<td></td>
<td>A23187†</td>
<td>290.4</td>
<td>468.3</td>
<td>228.4</td>
</tr>
<tr>
<td>Experiment 2</td>
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<td>—</td>
</tr>
<tr>
<td></td>
<td>10⁻³</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
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<td>204.0</td>
<td>115.1</td>
</tr>
<tr>
<td></td>
<td>A23187†</td>
<td>244.9</td>
<td>341.0</td>
<td>589.0</td>
</tr>
</tbody>
</table>

Ketamine did not induce release of histamine from basophils obtained from 14 different donors (fig. 2). In contrast, ketamine induced histamine release from lung mast cells in a concentration-dependent manner (P < 0.001). In 12 experiments, ketamine released a maximum of 6.2 (0.9) % of lung mast cell histamine content (P <
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FIG. 4. Effect of anti-IgE (0.3 µg ml⁻¹ in basophils, 1 µg ml⁻¹ in human lung mast cells (HLMC) and 3 µg ml⁻¹ in human skin mast cells (HSMC)), propofol and A23187 (0.2 µg ml⁻¹ in basophils and 1 µg ml⁻¹ in HLMC and HSMC) on the release of LTC₄ from human basophils (A) and lung mast cells (B), and of PGD₂ from skin mast cells (C) (mean (SEM) obtained in three experiments). ***P < 0.001 compared with spontaneous release.

Our results indicate that general anaesthetics induce histamine release selectively from mast cells and that mast cells isolated from different anatomical sites (lung parenchyma, skin tissue and heart fragments) can be useful to evaluate in vitro the histamine releasing capacity of general anaesthetics.

Previous studies have demonstrated that skin mast cells are exquisitely sensitive to atracurium, tubocurarine [33] and morphine [23, 34]. Our results extend these observation by demonstrating the existence of different types of heterogeneity. First, histamine-containing cells (basophil granulocytes and mast cells isolated from different anatomical sites) showed a remarkable degree of heterogeneity in response to the same type of general anaesthetic. In fact, we have demonstrated that peripheral blood basophils are essentially unresponsive to the wide spectrum of concentrations of general anaesthetics used in this study. Therefore, the in vitro model of basophil histamine release, which is potentially useful to detect patients who possess specific IgE against an
epitope present in neuromuscular blocking drugs [35], does not lend itself to the study of anaphylactoid reactions. There is also a remarkable degree of heterogeneity among mast cells obtained from different human tissues. For example, pharmacological concentrations of propofol induced histamine release from lung mast cells, whereas they had a more modest or no effect on skin and heart mast cells, respectively. Finally, we found that the general anaesthetics used in this study possess a remarkable degree of heterogeneity in their releasing capacity among different donors. The latter observation might be clinically relevant because it is well known that anaphylactoid reactions occur only in a small proportion of patients undergoing general anaesthesia. Therefore, the heterogeneity of the response of human FcRI+ cells—that is, the histamine-releasing capacity shown by different general anaesthetics and the degree of the response of mast cells obtained from different donors—might explain, at least in part, the wide spectrum of the anaphylactoid reactions observed in different patients after administration of these drugs.

In the present study, thiopentone induced a small amount of histamine release from skin mast cells. This is in apparent contrast to the findings reported by Hirshman and colleagues [12]. However, they used greater concentrations of thiopentone and their system to challenge skin mast cells differed from ours: they used fragments of infants foreskin, while we used mast cells enzymatically dispersed from skin obtained from adults, and there is evidence that the age of cell donors influences the release of human basophils [36] and of mast cells [19, 22].

Propofol [16, 17], ketamine [9] and thiopentone [7, 37] may induce haemodynamic alterations in man, caused presumably by histamine release in vivo [6, 7]. Histamine administration reproduces most of the metabolic and haemodynamic effects observed during anaphylactic/anaphylactoid reactions [13–15]. However, other mediators synthesized by human basophils and mast cells (LTC4 and PGD2) might contribute to these effects [38]. We have demonstrated that the general anaesthetics used in this study did not induce the de novo synthesis of LTC4 or PGD2 from human basophils and mast cells. This novel observation might indicate that histamine H1- and H2-blockers could be sufficient to prevent the anaphylactoid reaction caused by these drugs, as shown previously for morphine [39, 40].

The in vitro concentrations that we used include those usually found in the peripheral blood of patients undergoing general anaesthesia [32]. Therefore, the local concentrations of these drugs during or immediately after i.v. infusion might be relevant, considering also the variability in the releasing activity of cells from different donors and the variations of pharmacokinetics in different clinical conditions [32].

In conclusion, our results are the first systematic evaluation of the histamine-releasing activity of three commonly used i.v. anaesthetic agents using three different in vitro systems. We have demonstrated that these agents induce only histamine release selectively from mast cells. These findings might, at least in part, explain one of the mechanisms of anaphylactoid reactions caused by these drugs in some patients undergoing general anaesthesia. The experimental models described here might be useful to extend the present knowledge of the property of several anaesthetics to release histamine from human basophils and mast cells.

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