Thiobarbiturate-induced Histamine Release in Human Skin Mast Cells

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Human skin mast cell preparations were incubated with thiopental, thiamylal, methohexitol, and pentobarbital in concentrations ranging from $10^{-5}$ M to $10^{-3}$ M. Both thiopental- and thiamylal-induced dose-related histamine release, with thiamylal having a significantly greater effect than thiopental ($P < 0.05$). In contrast, incubation of skin mast cell preparations with the same concentrations of pentobarbital and methohexitol failed to increase histamine release above spontaneous levels at any concentration. The release of histamine by thiopental and thiamylal was not accompanied by the leakage of lactic dehydrogenase (LDH). Although a demonstration of histamine release in vitro is not proof that clinical symptoms are causally related to histamine release in vivo, methohexitol may be preferred as the induction agent in patients showing extreme sensitivity to histamine (asthmatic) or increased histamine reactivity (atopic). (Key words: Anesthetics, intravenous: methohexitol; pentobarbital; thiamylal; thiopental. Histamine: mast cells; release.)

Numerous review articles on drugs that release histamine from mast cells fail to list thiobarbiturates as histamine releasers.1–4 Yet Lorenz et al.5 measured increases in plasma histamine levels in humans after intravenous administration of thiopental. Moreover, generalized erythema, hypotension, and bronchospasm have been reported during their use.6–19 Although these symptoms are thought to be the result of discharge of histamine and other vasoactive and bronchoactive substances from mast cells, the mechanisms stimulating this release have been unclear. Many investigators have been unable to prove that the anaphylactoid reaction observed was immunologically mediated. In one-third of the cases reported by Clarke and Cockburn,7 patients had not previously received the drug.

Using human skin mast cell preparations, we determined if a series of commonly used barbiturates released histamine by a nonimmunological mechanism and if so what properties of the drug were important in this phenomenon.

Methods

This study was exempted from institutional review.

Human Foreskin Preparation

Freshly excised infant foreskins were placed in individual tubes (Corning® 25330) that contained Dulbecco’s Modified Eagle Medium® (Gibco Laboratories) with 1% Antimicrobic Solution® (Gibco Laboratories). All samples were processed within 4 h of excision. Each foreskin was spread and tacked on a Styrofoam® cutting board and divided into eight to 12 similarly sized rectangular portions with a blade. To increase the surface area for drug interaction, each piece was sectioned further into standardized 200-μm-width strands with a Sorvall TC-2® tissue sectioner as described by Tharp et al.6 Each sectioned piece was placed in a polypropylene tube and washed three times in 2 ml of buffer containing 0.1 mg/ml human serum albumin (HSA), 135 mM NaCl, 3.7 mM KCl, 5 mM Na3PO4, 5 mM dextrose, and 2 mM HEPES, pH 7.2 to remove support medium, tissue sectioning freezing compound, and blood.

Barbiturate-induced Histamine Release

After washing, the sectioned pieces were suspended in 1.0 ml of buffer with 2.5 mM calcium added and incubated for 30 min in a 37°C water bath. Tissue strands were resuspended by tapping the tubes every 10 min. The supernatant was aspirated from each tube, placed in a plastic sample cup, and frozen at −70°C until assayed for histamine concentration. This initial supernatant was used to determine spontaneous predrug exposure histamine release in each piece.

Sodium thiopental (Abbott, Chicago, Illinois), sodium thiamylal (Parke Davis, Morris Plains, New Jersey), sodium pentobarbital (Sigma, St. Louis, Missouri) and sodium methohexitol (Eli Lilly, Indianapolis, Indiana) were diluted to final concentrations ranging from $10^{-5}$ M to $10^{-3}$ M in Ca++ HSA buffer.

Each sectioned piece then was resuspended in 1.0 ml of test drug or buffer (control) and incubated for an additional 30 min at 37°C with resuspension by tube agitation every 10 min. Each drug concentration was tested.


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induced histamine release, and residual histamine. All results are expressed as per cent of net histamine release, which was calculated by subtracting per cent of spontaneous histamine release from per cent of drug-induced histamine release. Results were discarded if the spontaneous histamine release was greater than 15% in skin mast cells.

**LDH Leakage into the Supernatant**

To determine if thiopental and thiamylal damaged cell membranes and resulted in leakage of lactic dehydrogenase (LDH) into the supernatant, LDH was measured using a spectrophotometer (Gilford, Oberlin, Ohio) in six separate foreskin experiments (three each). Foreskins were each divided into three pieces, sectioned as previously described, and incubated in buffer for 30 min at 37°C. LDH was measured and assigned a relative activity of 100%. One of the three pieces was incubated in buffer (negative control); one in water, which lysed cells (positive control); and one in drug (10^{-3} M) for an additional 30 min, and LDH and histamine were measured. LDH was expressed as per cent change from the LDH measured after the first incubation.

**Statistical Analysis**

Data were compared by a one-way analysis of variance and a Student-Neuman-Keuls test for multiple comparison. The level of statistical significance used was $P < 0.05$.

**Results**

In skin mast cell preparations in these studies, spontaneous histamine concentrations ranged from 21 to 38 ng/ml and total histamine concentrations ranged from 110 to 250 ng/ml. Mean spontaneous histamine release was 10.9 ± 0.33% (mean ± SEM).

Both thiopental and thiamylal induced dose-related histamine release in skin mast cell preparations (fig. 1). Histamine release by thiamylal was significantly greater ($P < 0.05$) than by thiopental at drug concentrations ranging from 3.3 × 10^{-2} M to 10^{-3} M. In contrast, incubation of skin mast cell preparations with the same concentrations of pentobarbital and methohexital failed to induce release of histamine above spontaneous levels at any concentration (fig. 1).

Neither thiopental nor thiamylal induced leakage of LDH into the supernatant, whereas incubation with water increased LDH activity in the supernatant 200 ± 27% ($P < 0.05$).

**Reproducibility of Data**

There was little variability in per cent histamine release when two pieces from the same foreskin were incubated in duplicate. The supernatant was aspirated and frozen until assayed. This supernatant was used to determine the drug-induced histamine release.

To determine the residual histamine content of each sectioned piece, the tissue from each tube was placed in 3 ml of Ca^{2+} HSA buffer, boiled for 10 min, and then homogenized with a Polytron® homogenizer. After centrifugation at 800 g × 5 min, the supernatant was aspirated and frozen until assayed.

**Histamine Determinations**

Concentrations of histamine in supernatants were determined by the automated fluorometric method of Siragianian17 (Alpkem Histamine AutoAnalyzer®, Clackamas, Oregon). The sensitivity was 2 ng/ml of histamine. Assay specificity was confirmed by incubation with diamine oxidase (Sigma, St. Louis, Missouri), which eliminated all detectable histamine. A standard curve was generated with histamine solutions (Sigma, St. Louis, Missouri) of known concentration.

Spontaneous and drug-induced histamine release were calculated as a per cent of the total histamine present. The total histamine in each sectioned piece of foreskin was calculated by adding the histamine concentrations in the supernatants for spontaneous histamine release, drug-induced histamine release, and residual histamine. All results are expressed as per cent of net histamine release, which was calculated by subtracting per cent of spontaneous histamine release from per cent of drug-induced histamine release. Results were discarded if the spontaneous histamine release was greater than 15% in skin mast cells.
with the same drug concentration. Each drug concentration was tested in duplicate in the same foreskin. In the same foreskin at the same drug concentration, the difference in actual percent histamine release in the two pieces varied from 0.2 to 1.6%. When pieces from different foreskins were tested with similar drugs and concentrations either on the same day or on different days, the percent histamine release was more variable. In five different foreskins treated with thiopental (10⁻⁵ M) tested on either the same day or on different days, histamine release varied from 7.0 to 15.0%, with a mean of 12.5% and a standard deviation of 9.2%. In six different foreskins treated with thiamyllal (10⁻³ M) tested on either the same day or on different days, histamine release varied from 13.5% to 24.9%, with a mean of 20.3% and a standard deviation of 4.6%. Because of the variability in histamine release between foreskins, each skin was sectioned into eight to 12 pieces and incubated with a whole range of drug concentrations and different drugs in any one experiment.

Discussion

Thiopental and thiamyllal in concentrations used in clinical anesthesia induced significant histamine release in human skin mast cell preparations. In contrast, pentobarbital and methohexital, at all concentrations tested, failed to induce histamine release in this preparation.

Barbiturates may cause histamine release by a degradation mechanism that leaves the cell itself intact or by destruction of the cell membrane, in which case LDH activity in the supernatant would increase. It is unlikely that histamine release by thiobarbiturates resulted from drug-induced cell membrane damage, since LDH activity in the supernatant did not increase in preparations incubated with thiobarbiturates, whereas histamine levels did.

Barbiturates may cause mast cell degranulation by displacing histamine from binding sites or by changing the biophysical characteristics of granule or cell membranes. Drug characteristics that may be relevant include lipid solubility, degree of ionization, high concentration of an acidic drug, or a specific molecular configuration of the drug itself. We selected a series of barbiturates with differing structures, lipid solubilities, and pKₐ's in an attempt to understand this phenomenon (table 1). Histamine release by barbiturates does not depend on high lipid solubility, since methohexital, which is far more lipid-soluble than thiopental, did not release histamine. Similarly, since neither methohexital nor pentobarbital released histamine even in millimolar concentrations, histamine release by barbiturates does not reflect a nonspecific effect of a high concentration of barbituric acid. Although both thiopental and thiamyllal have low pKₐ's, and therefore high concentrations of the ionized form of the drug, the ionized moiety is not in itself the culprit since a millimolar concentration of pentobarbital provides as much ionized barbiturate as 3.3 × 10⁻⁴ M thiopental and thiamyllal, which release histamine, whereas pentobarbital 10⁻⁵ M does not. The structures of pentobarbital and thiopental are very similar except for one sulfur replacing one oxygen in the thiopental molecule, yet thiopental releases histamine and pentobarbital does not. This suggests that the sulfur atom is important in barbiturate-induced histamine release. Our data with thiamyllal and data presented by Paton on disoithioareas are also consistent with this view.

Histamine release occurs in our mast cell preparations at concentrations that are seen in clinical practice. Plasma thiopental concentrations, after a bolus dose that induces anesthesia, average 2.48 × 10⁻⁴ M (28 mg/l). The initial concentration of thiopental in the syringe is about 10⁻¹ M. If 300 mg of thiopental is injected into a 60-kg human (with circulating blood volume of 5 l), an initial plasma concentration in the 2 × 10⁻⁴ M range would result when most of the drug is still confined to the circulating blood volume. Therefore, mast cells along the vasculature are exposed to thiopental concentrations somewhere between 10⁻¹ M and 2 × 10⁻⁴ M. Thiamyllal plasma concentrations are similar to thiopental.

A demonstration of histamine release by thiobarbiturates "in vitro" is not proof that all or any clinical signs attributed to the drug are causally related to histamine release in vivo. Plasma levels of histamine reported in five patients 20 s after a 5 mg/kg intravenous bolus dose of thiopental averaged 5 ng/ml and were associated with no changes in heart rate and blood pressure, suggesting that in some patients histamine release by thiopental is relatively small and is therefore of little clinical importance.

However, life-threatening reactions have been reported with these agents, and in more than one-third of a reported 100 cases, no plausible explanation on the basis of an immunologic mechanism was apparent. Moreover, classes of patients (atopic) exist that demonstrate greater "histamine releasability" than nonatopic controls, and patients with airway hyperreactivity demonstrate extreme airway sensitivity to many mediators including histamine. In such patients, methohexital may well be the barbiturate of choice for induction of anesthesia.
This in vitro human foreskin preparation is useful in studying drug-induced histamine release. Previous studies on this subject have used cell preparations from animals or human basophils. However, recent studies show marked functional differences between mast cells of different species, and studies in our laboratory show functional differences between human mast cells and human basophils with respect to drug-induced histamine release. The human foreskin preparation contains high concentrations of mast cells, which degranulate in the presence of drugs such as morphine. Histamine release is not accompanied by cell membrane damage histologically and occurs in this preparation at lower concentrations than are seen in rat mast cells.

In summary, using human skin mast cell preparations, we have demonstrated histamine release by thiopental and thiamylal, but not by pentobarbital and methohexitol at the same concentrations. Our data show that the sulfur analogues of the barbiturates induce histamine release in human mast cell preparations and suggest that in patients showing extreme sensitivity to histamine (asthmatics) or increased histamine releasability (atopics), methohexitol may be the induction agent preferred.

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