

Leukocyte Histamine Release to Thiopental

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More than 50 reports of presumed anaphylactic shock due to thiopental have been reported in the past 25 years.¹ Although the mechanism of these adverse reactions is unclear,¹ the clinical features (generalized erythema, hypotension, edema, and bronchospasm²) are thought to result from massive discharge of histamine and other vasoactive substances from basophils and mast cells. Because the patient may require further anesthesia at some later date, it is important to identify the agent causing mediator release and the mechanism of the adverse reaction. The amount of histamine released from the patient's own peripheral blood leukocytes and from the serum incubated with control leukocytes after challenge with varying concentrations of agents received during the anesthesia^{3,4} may identify the causative agent and the mechanism involved.

The case report demonstrates leukocyte histamine release to extremely low concentrations of thiopental with no evidence for an immunoglobulin E (IgE) mediated type 1 hypersensitivity mechanism.

REPORT OF A CASE

A 64-year-old, 85-kg man was admitted to a hospital for excision of a groin mass. Past medical history included asthma for many years controlled by oral theophylline, mild hypertension requiring no treatment, alcohol abuse, and a 90-pack -year cigarette smoking history up to 12 years prior to the present admission. Previous surgeries included tonsillectomy as a child, excision of a neck mass as a teenager, and closed reduction of the left tibia two years previously. Details of these anesthetics are unknown. One month prior to the present admission, the patient underwent a right inguinal hernia repair under subarachnoid block anesthesia, at which time the left groin mass was discovered.

One day preoperatively, expiratory rhonchi were present for which an intravenous infusion of aminophylline was begun, which was continued through induction of anesthesia. The plasma theophylline level prior to induction was 7 µg/ml. Analysis of arterial blood gases, while breathing room air was within normal limits. The electrocardiogram showed changes consistent with mild ischemia. No history of drug allergy was obtained.

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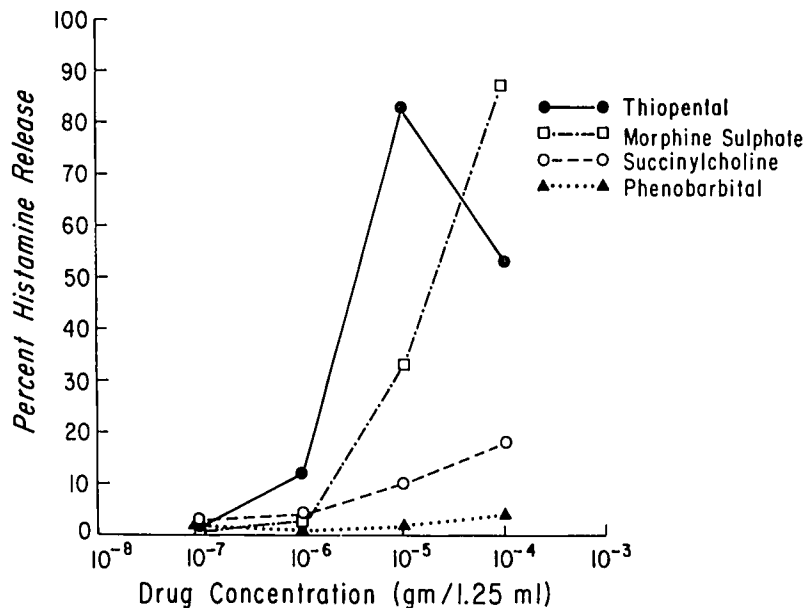
On the morning of the scheduled surgery, the chest was clear to auscultation. One and one-half hours before anesthesia, 6 mg morphine and 25 mg promethazine were administered im. Arterial blood pressure was 150/90 mmHg and heart rate 90/min. A subarachnoid injection of tetracaine, 12 mg, with epinephrine was administered. Five minutes later, arterial blood pressure was 140/90 mmHg. Prophylactic ephedrine (25 mg) was given im and 100 per cent oxygen was administered via face mask. After 20 min loss of sensation was experienced up to T-12 and spinal anesthesia was judged inadequate for surgery. Thiopental, 300 mg, was administered iv over a 30-second period followed by two additional aliquots of 100 mg over a period of two minutes. Because of difficulty with ventilation, an oral airway was inserted. Arterial blood pressure was 100/80 mmHg and heart rate 120/min immediately after the first dose of thiopental. Three minutes later, 100 mg succinylcholine was administered iv; however, providing adequate ventilation was difficult with an inspired concentration of 3 per cent halothane in oxygen. Intubation of the trachea was performed immediately, at which time edema of the false vocal cords was observed. Expiratory rhonchi were heard bilaterally. A bright red blush was observed over the face and upper body. One minute later, the ECG showed a heart rate of 120/min, arterial blood pressure was unobtainable and femoral pulses were not palpable. Halothane was discontinued. Ephedrine, 25 mg, and then epinephrine, 1 mg, were administered iv. The ECG showed sinus tachycardia with marked ST depression, followed by ventricular fibrillation 30 s later. External cardiac massage was instituted and defibrillation was successful on the third attempt. Calcium chloride (250 mg) and sodium bicarbonate (50 mEq) were given at the onset of ventricular fibrillation. Arterial blood pressure (systolic) was 65 mmHg immediately following defibrillation. An infusion of dopamine was started because of persistent hypotension. The erythema, extending from the level of the iliac crests to the face, persisted for 15 to 20 min and gradually faded over the next hour. Below the iliac crests, the skin was cyanotic and blotchy. Over the next hour, expiratory time was prolonged and CO₂ retention persisted. The patient was transferred to the surgical intensive care unit one h and 10 min after the arrest. The patient did not regain consciousness over the next two weeks and ventilation was controlled for 10 days. On the seventh and tenth day after the arrest, arterial blood was obtained for immunologic studies. The patient died 14 days after the arrest. Permission for postmortem examination was denied.

LEUKOCYTE HISTAMINE RELEASE METHOD

Leukocyte histamine release measurements were made on 40 ml of blood obtained from the patient and 40 ml of blood obtained from five control subjects. Blood was obtained from the patient on the seventh day after the anaphylactoid reaction for histamine release to thiopental and on the tenth day following the anaphylactoid reaction for passive sensitization of control cells. All cells were tested for histamine release on the day on which they were drawn.

The blood was transferred in 10-ml aliquots to plastic tubes containing 2.5 ml of a mixture of dextran 3 per

FIG. 1. Leukocyte histamine release in cells obtained from the patient.



cent and glucose 3 per cent. (Nutritional Biochemical Corporation, Cleveland, Ohio) and 1 ml 0.1 M ethylenediamine tetra-acetate (EDTA). The mixture was allowed to sediment at room temperature for 60 to 90 min until a clear interface developed between the erythrocyte and plasma layers. The leukocyte layer was then drawn off and washed in cold (4°C) Tris-saline-EDTA buffer as described by May *et al.*⁵ and the cells counted in a Coulter counter. Washing of the cells removes complement.

Histamine release from leukocytes was carried out according to the method of May *et al.*⁵ The drugs tested for histamine release included thiopental, morphine, succinylcholine, and phenobarbital. Drug solutions were made up in Tris-saline buffer containing magnesium and calcium as described by May *et al.*⁵ The drug concentrations used to induce histamine release ranged from 0.08–800 µg/ml in the final leukocyte suspensions. Preliminary experiments indicated that none of the drugs interfered with the fluorometric analysis of histamine under the conditions employed. Histamine concentrations were determined by the fluorometric method of Shore *et al.*⁶

One set of studies investigated the ability of the previously mentioned drugs to induce a direct dose-related histamine release from the patient's leukocytes. The second set of studies investigated the ability of the patient's serum to passively sensitize *in vitro* leukocytes from five individuals. The passive sensitization procedure used was that described by Levy and Osler.⁴ Passive sensitization was carried out at a final cell concentration of 1×10^7 leukocytes/ml using the hypersensitive serum at a 10 per cent concentration and incubating the cells at 37°C for 30 and 60 min. Thiopental, in the same concentrations,

was added to the leukocyte aliquots and histamine release measured by methods described. Histamine release in the absence and in the presence of drugs was expressed as a percentage of total cellular histamine content.

RESULTS

In the peripheral leukocytes of the patient, thiopental caused histamine release in concentrations as low as 10^{-6} g/1.25 ml, which is a subanesthetic concentration^{7,8} (fig. 1). Phenobarbital elicited little or no histamine re-

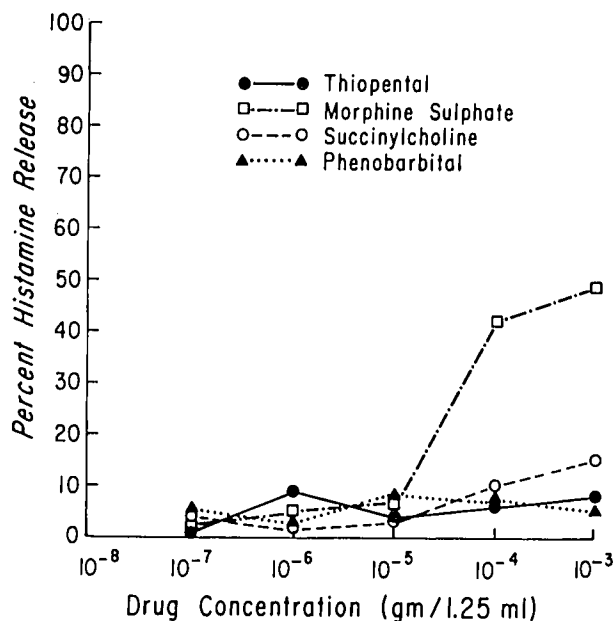


FIG. 2. Leukocyte histamine release in cells obtained from control subject 1.

TABLE 1. Percentage of Leukocyte Histamine Release by Thiopental (10^{-5} M)

	Direct Release	After Passive Sensitization*
Patient	82.5	—
Control 1†	4.8	10.0
2	2.7	4.7
3	10.5	9.3
4	17.0	0.0
5	10.0	10.2

* Sixty-min incubation of leukocytes with serum from patient.
† Atopic (allergic rhinitis).

lease at a 1000-fold greater concentration. In contrast, thiopental elicited little or no histamine release in the control cells shown in figure 2 and from the cells obtained from four additional individuals until concentrations of thiopental of 10^{-3} g/1.25 ml had been reached. Morphine sulphate, a known histamine liberator, released histamine from both the cells of the patient and the control. Succinylcholine, a weak histamine liberator also released histamine from both the patient's cells and the cells of the control but only in very high concentrations (figs. 1 and 2).

Incubation of the serum of the patient obtained 10 days after the anaphylactoid reaction with the cells from five control subjects elicited little or no histamine release (table 1); an example is shown in figure 3. Passive transfer of a thiopental-specific factor from the serum of the patient to the cells of the five control subjects did not occur (table 1, fig. 3).

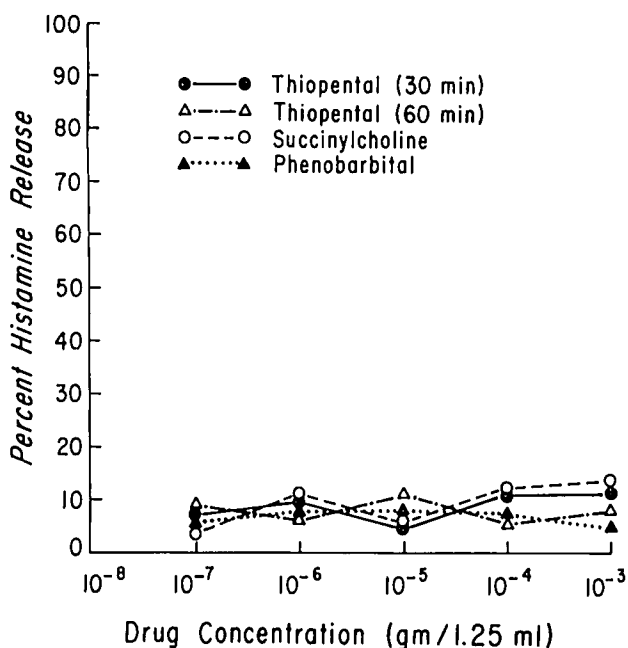


FIG. 3. Passive sensitization of cells from control subject 1 incubated with serum obtained from the patient.

DISCUSSION

The clinical features of generalized erythema, profound hypotension, severe bronchospasm, and laryngeal edema conform to a classic anaphylactoid reaction. Leukocyte histamine release to concentrations of thiopental achieved in blood identifies thiopental as the offending agent. The absence of significant histamine release after passive sensitization is evidence against an IgE mediated mechanism and is suggestive that the anaphylactoid reaction resulted from a direct effect of thiopental on mast cells and basophils.

The confirmation of hypersensitivity to thiopental has been difficult in the past.⁹ Direct intravenous challenge with thiopental is hazardous and the value of skin testing questionable in other than allergic reactions to penicillin.¹⁰ Estimation of plasma complement C3 consumption and conversion in sequential blood samples taken at intervals over the first 24 h following an adverse reaction has been advocated by Watkins *et al.*¹¹ as a simple and convenient method for assessing hypersensitivity reactions. This method does not identify the agent causing the reaction. It does not identify IgE mediated type 1 hypersensitivity reactions nor direct pharmacologic release of mediators, as both mechanisms do not involve the complement system. Moreover, this test cannot be used to predict a reaction to thiopental in a patient with a prior adverse reaction to the drug. Serial measurement of serum IgE^{12,13} also has been advocated as a test to detect hypersensitivity reactions to drugs. This test, too, does not identify the offending agent and cannot be used to predict a drug reaction in a patient labelled sensitive to a drug. Passive transfer of cutaneous reactivity (Prausnitz-Küstner test) has long provided the major approach for the diagnosis of hypersensitivity reactions of the immediate type.⁴ This test is accurate and has been used to diagnose thiopental hypersensitivity¹⁴ but involves the exposure of a volunteer to the possibility of hepatitis. Because thiopental has a direct toxic effect on lymphocytes,⁹ lymphocyte transformation tests¹⁰ give unreliable results. The indirect basophil degranulation test described by Shelly¹⁵ and used by Fox *et al.*¹⁶ partially duplicates passive transfer in a test tube. The serum from allergic patients may passively sensitize rabbit basophils to the drug in question. Addition of the drug to the preparation results in degranulation, which is said to be proportional to the hypersensitivity.¹⁵ This test has the advantage of possibly identifying the offending agent if the reaction is IgE mediated but has the disadvantage of not identifying the agent if the mechanism is direct release of mediators. Quantitation is difficult with this test and use of cells from a species other than humans is not desirable in view of the known species variations with respect to sites of storage, nature, quantity, and tissue reactivity to those compounds whose release is being measured.³

The leukocyte histamine release test simulates the anaphylactoid reaction in the test tube using the patient's own cells. It not only identifies the offending agent,^{17,18} but it establishes concentration of the drug needed to release histamine. This test also may differentiate direct non-immunologic release of histamine from an immunologic mechanism. Measurement of histamine is a standard laboratory technique.⁶

We cannot totally rule out an allergic mechanism in our patient although it is unlikely. This patient probably received thiopental two years previously, and the failure to sensitize control cells passively may reflect a failure of the patient's antibody to bind with the control cells.

If a reaction results from direct histamine release rather than from immunologic mechanisms, the reaction is related to volume, concentration, rate of injection¹⁹ as well as to factors in the mast cells and basophils regulating mediator release.²⁰ Subsequent administration of the drug may be safe.²¹

The release of chemical mediators from human leukocytes and lung tissue is modulated by agents that alter the cellular levels of cyclic 3',5'-adenosine monophosphate (cyclic AMP).¹⁹ Enhancement of release is associated with depletion of the level of cyclic AMP in cells. A decrease in circulating catecholamines from the adrenal medulla following the subarachnoid block may have enhanced mediator release in our patient. Conversely, theophylline ought to inhibit mediator release²⁰ but the serum theophylline level was only 7 µg/ml (therapeutic level 10 to 20). Moreover, atopic patients as a group demonstrate greater leukocyte histamine release to some non-specific substances than do nonatopic controls.^{22,23} Lorenz *et al.*²⁴ have demonstrated release of histamine following injection of thiopental even in normal subjects. In their studies, plasma histamine levels correlated well with severity of symptoms in patients experiencing symptoms. One third of patients studied by Clark *et al.*²⁵ and one half of patients studied by Fisher,¹⁹ who had reactions to intravenous induction agents, had no history of prior drug exposure. Watkins in a recent review states that "relatively few anaphylactoid reactions to iv substances are antibody mediated."¹

In severe anaphylactoid reactions to anesthetic agents, it is important to identify the causative agent in order to avoid its subsequent use. Measurement of leukocyte histamine release appears useful in this regard.

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