Halothane Selectively Inhibits Bradykinin-induced Synovial Plasma Extravasation

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Background: In vitro studies demonstrate that halothane, but not isoflurane, inhibits bradykinin-induced calcium currents and prostacyclin release in cultured endothelial cells. Because bradykinin is an important endogenous mediator of inflammation, we assessed the effects of halothane, isoflurane, and pentobarbital on plasma extravasation, a component of tissue inflammation induced by bradykinin, in rats.

Methods: We anesthetized 23 rats with halothane (0.8 or 1.3 minimum alveolar concentration [MAC]), isoflurane (1.3 MAC), or pentobarbital (total of 85 mg/kg intraperitoneally). Their tracheas were intubated and their lungs mechanically ventilated. After intravenous administration of Evans blue dye, we infused normal saline followed by bradykinin or platelet-activating factor, another inflammatory mediator, intravenously via needles placed in the knee joint. We collected perfusate and estimated extravasation by measuring dye in the perfusate using spectrophotometry.

Results: Bradykinin increased plasma extravasation eight to ninefold above baseline in both pentobarbital- and isoflurane-anesthetized rats. In contrast, bradykinin-inhibited plasma extravasation at 0.8 MAC and 1.3 MAC of halothane was approximately 40% (P < 0.01) and 15% (P < 0.001), respectively, of that in pentobarbital- and isoflurane-anesthetized rats. Baseline plasma extravasation was lower in rats anesthetized with either concentration of halothane compared with pentobarbital or isoflurane (all P < 0.001). Platelet-activating factor-induced plasma extravasation was similar for all anesthetics.

Conclusion: Halothane, but not isoflurane or pentobarbital, inhibits both baseline and bradykinin-induced peripheral plasma extravasation, demonstrating that volatile anesthetics differentially modulate this important component of inflammation. (Key words: Anesthetics, intravenous; pentobarbital.

Anesthetics, volatile; halothane; isoflurane. Inflammation: plasma extravasation. Inflammation, mediators: bradykinin; platelet-activating factor.)

SURGICAL trauma causes release of inflammatory mediators, including bradykinin, serotonin, histamine, arachidonic acid metabolites, and numerous other compounds. These mediators elicit various components of the inflammatory response, such as pain, erythema, and plasma extravasation (edema). The effects of most volatile anesthetics on inflammatory mediator actions have not been studied to date. The exception is diethyl ether, which has been shown in rats to increase histamine-induced, but not serotonin-induced, cutaneous vascular permeability compared with that in unanesthetized animals.7 Several studies have assessed the effects of volatile anesthetics on signal transduction mechanisms, such as interactions with G-proteins and intracellular calcium regulation.2-6 A few of these reports studied volatile anesthetic effects on the vasomotor actions of bradykinin, serotonin, and other inflammatory mediators.7-10 Recently, Loeb and colleagues reported that halothane (0.5 mm, equal to 1.5 vol%), but not isoflurane, selectively inhibits bradykinin-induced calcium currents and prostacyclin production in vitro in cultured bovine aortic endothelial cells, and that inhibition of prostacyclin release may result in the decreased vasodilation observed with halothane versus isoflurane.5,11 However, bradykinin-induced prostaglandin release is also important in local inflammatory processes.12 Studies in our laboratory have demonstrated that sympathetic terminal synthesis and release of mediators, such as prostaglandins and purines, mediate bradykinin-induced synovial plasma extravasation and that sympathectomy or intraarticular indomethacin inhibits this process.13-15

No previous studies have measured the in vivo effect of volatile anesthetics on the peripheral inflammation or injury produced by surgical procedures. The current study determined whether halothane can modulate, in vivo, plasma extravasation, a component of bradykinin-
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induced peripheral inflammation, in rat knee-joint synovium. The effect of halothane anesthesia on plasma extravasation was compared with that of pentobarbital and isoflurane. We also tested the effects of these three anesthetics on plasma extravasation induced by platelet-activating factor (PAF), which, unlike bradykinin, induces extravasation by a non–prostaglandin-mediated, non–sympathetic terminal–dependent mechanism.16

Materials and Methods

Animals

With approval of the Committee on Animal Research of the University of California, San Francisco, we studied 23 male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing 300–450 g. Rats were housed under controlled lighting conditions (lights on 6:00 AM to 6:00 PM), with food and water available ad libitum until the morning of study.

Anesthetic Protocol

Rats were prospectively randomized to receive pentobarbital, isoflurane or halothane anesthesia. Pentobarbital produces little effect on plasma extravasation compared with that in unanesthetized animals1 and therefore this anesthetic group served as the control. Rats selected for pentobarbital anesthesia received sodium pentobarbital (Anpro, Arcadia, CA) 65 mg/kg intraperitoneally for induction of anesthesia and an additional dose of 20 mg/kg at 40 min. The remaining animals were anesthetized by inhalation of isoflurane or halothane in a plastic chamber. All tracheas were intubated under direct vision with a 5.1 cm 16-G intravenous catheter and normocapnia achieved with mechanical ventilation (peak inspiratory airway pressures ≤ 20 mmHg).

Airway gas concentrations were continuously monitored with an infrared analyzer (CapnoMac Ultima, Datex Instrumentarium, Helsinki, Finland) as previously described.17 Normothermia was thermostatically maintained using a heat lamp and a rectal temperature probe. All rats received an intraperitoneal injection of 0.9% saline (10 ml/kg) to counter fluid loss due to mechanical ventilation. Isoflurane (Ohmeda, Murray Hill, NJ) or halothane (Ohmeda) anesthesia was maintained in oxygen to achieve an end-tidal concentration equal to 1.3 minimum alveolar concentration [MAC] (isoflurane 1.75 vol% and halothane 1.50 vol%) as determined in our laboratory for this strain. An additional group were maintained with halothane in oxygen at an end-tidal concentration equal to 0.8 MAC (0.92 vol%). Plasma extravasation studies were preceded by a 15-min period of anesthetic equilibration for pentobarbital and a 60-min equilibration period for isoflurane and halothane.

Plasma Extravasation

After anesthetic equilibration, we injected into a tail vein Evans blue dye (50 mg/kg in a volume of 2.5 ml/kg; Sigma Chemical, St. Louis, MO), a marker for plasma protein extravasation. The knee-joint capsule was excised by exposing the overlying skin, and a 30-G needle was inserted into the joint and used to infuse 0.9% saline solution (fig. 1). The infusion rate (250 μl/min) was controlled by a Sage Instruments Syringe pump (model 341B, Orion Research, Boston, MA). A 25-G needle, also inserted into the joint space, permitted extraction of fluid at 250 μl/min, controlled by another Sage Instruments Syringe pump (model 351). Perfusion fluid was collected in separate samples at 5-min intervals over a total collection period of 40 min. Evans blue dye concentration in each aliquot was determined by spectrophotometric measurement of absorbance at 620 nm. Absorbance is linearly related to dye concentration.18 Baseline plasma extravasation was measured by perfusing the saline solution intraarticularly and collecting three perfusate samples during the first 15 min of the plasma extravasation study period. Bradykinin (160 nm; Sigma) or PAF (100 nm; Sigma) was perfused into the joint, and the perfusate was collected and analyzed for dye concentration.

Perfusion flow rate: 250 μl/min

Fig. 1. Method to determine synovial plasma extravasation in the rat knee joint. After intravenous administration of Evans blue dye, saline followed by bradykinin was perfused intraarticularly, and perfusate was collected and analyzed for dye concentration.

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then added to the perfusion fluid for the remaining 25 min. The administered concentrations of these agonists were based on results from previous dose–response studies in our laboratory.13 Plasma extravasation with saline joint perfusion (baseline) followed by drug (bradykinin or PAF) joint perfusion was averaged at each 5-min interval. Each rat knee was considered as a separate n value. Rats were then killed by a lethal injection of pentobarbital and bilateral thoracotomy.

Data Analysis
Differences in plasma extravasation for the different treatment groups were assessed by one factor analysis of variance or two-tailed t test, as appropriate; Fisher’s test was used for post hoc analysis among treatment groups. A P value ≤ 0.05 was considered statistically significant.

Results
We discarded data from rats where we found physical damage of the knee joint or inflow and outflow mismatch (detectable by presence of blood in perfuse and high baseline plasma extravasation or knee-joint swelling due to improper needle placement). This resulted in a total of 36 joints, rather than the anticipated 46, from the 23 rats studied. For each anesthetic group, there was no significant difference in plasma extravasation between rats with both knees perfused versus one knee.

Baseline Plasma Extravasation
For each anesthetic group, there was no significant time-dependence among the three baseline measurements (at 5, 10, and 15 min) and, therefore, baseline plasma extravasation is reported as the average ± SEM of extravasation values at these three baseline time intervals. Baseline plasma extravasation was similar in rats anesthetized with pentobarbital (total dose 85 mg/kg intraperitoneally, 0.033 ± 0.003 absorbance units) or isoflurane (1.3 MAC, 0.035 ± 0.002 absorbance units), whereas halothane-anesthetized rats had significantly lower baseline plasma extravasation at both 0.8 MAC (0.015 ± 0.002) and 1.3 MAC (0.009 ± 0.001) (P < 0.001, by analysis of variance, for both halothane concentrations versus isoflurane and pentobarbital) (fig. 2). Halothane at 1.3 MAC resulted in significantly less baseline plasma extravasation than 0.8 MAC halothane (P < 0.01, t test).

Bradykinin-induced Plasma Extravasation
The time courses for bradykinin-induced plasma extravasation are shown in figure 3 for rats anesthetized with either pentobarbital (85 mg/kg), isoflurane (1.3 MAC), or halothane (1.3 MAC or 0.8 MAC). Bradykinin (160 nm) perfused intraarticularly (arrow in fig. 3) increased plasma extravasation with a similar time course in all of the anesthetic groups. Maximum plasma extravasation was observed 15 min after addition of bradykinin and remained at this level for at least 10 min. Therefore, comparison of plasma extravasation between the anesthetic groups is reported as the average ± SEM of the extravasation values at 15, 20 and 25 min after the addition of bradykinin.

Intraarticular bradykinin induced maximal plasma extravasation eight- to ninefold greater than baseline in pentobarbital- and isoflurane-anesthetized rats, producing absorbance units of 0.300 ± 0.038 for pentobarbital and 0.266 ± 0.044 for isoflurane (fig. 3). Bradykinin induced significantly less plasma extravasation in the 3 MAC halothane-anesthetized rats (0.046 ± 0.018), which is 15% and 17% of the plasma extravasation in pentobarbital-and isoflurane-anesthetized rats, respectively (both P < 0.001). The effect of halothane was dose-related as evidenced by findings in rats anesthetized with 0.8 MAC halothane. Bradykinin-induced plasma extravasation at 0.8 MAC halothane (0.119 ± 0.018) exceeded extravasation at 1.3 MAC halothane (P < 0.001, t test) and was 40% and 45% of

![Fig. 2. Baseline synovial plasma extravasation for the four anesthetized groups: pentobarbital 85 mg/kg intraperitoneally, total dose (PB) (n = 10); isoflurane 1.3 MAC (ISO) (n = 10); halothane 0.8 MAC (HAL) (n = 6); and halothane 1.3 MAC (HAL) (n = 10). * P < 0.001 by analysis of variance for 0.8 HAL or 1.3 HAL versus ISO and PB. ** P < 0.01 by t test for 0.8 HAL versus 1.3 HAL. Values are means ± SEM.](image)

![Fig. 3. Time courses for bradykinin extravasation for the four anesthetized groups: pentobarbital (PB), isoflurane (ISO), halothane 0.8 MAC (HAL), and halothane 1.3 MAC (HAL). (0.005 nm) (n = 5). Values are means ± SEM.](image)
the plasma extravasation in pentobarbital- and isoflurane-anesthetized rats, respectively (both \(P < 0.01\)).

**Platelet-activating Factor-Induced Plasma Extravasation**

The time courses for PAF-induced plasma extravasation for pentobarbital, isoflurane and halothane (1.3 MAC) are shown in figure 4. PAF-induced plasma extravasation did not differ among the three anesthetic groups \((P > 0.05, \text{by analysis of variance})\) and is similar to extravasation produced by bradykinin in pentobarbital and isoflurane-anesthetized rats.

**Discussion**

The current study shows that halothane, but not pentobarbital or isoflurane, inhibits bradykinin-induced synovial plasma extravasation in rats. Halothane's effect is concentration-dependent (fig. 3). We previously demonstrated that bradykinin-induced synovial plasma extravasation requires intact sympathetic terminals and is inhibited by cyclooxygenase inhibitors.\(^{14}\) Bradykinin stimulates receptors linked to the phosphatidylinositol second-messenger system, which activates both protein kinase C and arachidonic acid metabolism.\(^{19,20}\) The component of the phosphatidylinositol second-messenger system involved in bradykinin-induced prostaglandin synthesis and release from sympathetic terminals is not known. Although recent studies have demonstrated halothane's ability to inhibit various signal-transduction components,\(^ {24-27} \) the mechanism for halothane's inhibition of bradykinin-induced plasma extravasation is unclear.

Halothane selectively inhibits bradykinin-induced plasma extravasation as demonstrated by its lack of effect on PAF-induced plasma extravasation. PAF-induced plasma extravasation differs from that of bradykinin in that PAF does not require the presence of neuronal terminals but appears to produce plasma extravasation by a direct action on vascular endothelium.\(^ {16} \) Therefore, halothane most likely inhibits plasma extravasation, not by a direct vascular effect, but rather at a more proximal site (i.e., the sympathetic efferent nerve terminal). Unlike bradykinin-induced plasma extravasation, prostaglandins do not mediate PAF-induced plasma extravasation, as evidenced by a lack of inhibition by indomethacin.\(^ {13} \)

The capacity of halothane to inhibit the sympathetic nervous system does not explain our results. Sympathetic neuron terminal synthesis and release of prostaglandins stimulated by bradykinin is thought to result from local stimulation of bradykinin receptors on the
sympathetic terminal and does not involve neuronal excitation of sympathetic neurons. In fact, norepinephrine, which is released from stimulated sympathetic effector neurons, inhibits plasma extravasation. In addition, cardiovascular changes are not likely explanations for our results. We previously demonstrated that plasma extravasation is independent of blood pressure over a wide range of pressures. Also, PAF-induced plasma extravasation was similar for all three anesthetics, suggesting that any differential cardiovascular effects of the anesthetics do not noticeably affect plasma extravasation in this model.

Consistent with our current in vitro findings on the capacity of halothane to inhibit bradykinin-induced plasma extravasation and the in vitro study demonstrating halothane inhibition of bradykinin-stimulated prostacyclin release from endothelial cells, halothane inhibits bradykinin-induced vasodilation in both in vivo pulmonary vasculature and in vivo systemic vasculature. Isoflurane was not tested in these models. Halothane, but not isoflurane, inhibits the vasoconstrictive effects of serotonin, histamine and prostaglandin E2 on human coronary arteries in vitro, suggesting that halothane can inhibit signal transduction mechanisms of other inflammatory mediators as well. However, other studies demonstrate that isoflurane can inhibit serotonin-induced vasoconstriction.

In apparent contrast to our current findings, halothane (2.5 vol%) increases capillary permeability in the ex vivo perfused rabbit lung model after oxidant injury. Similarly, halothane (1 vol%) increases in vivo alveolar-capillary barrier permeability in the presence of an inspired oxygen fraction of 0.99 but not 0.30. Both halothane and isoflurane potentiate oxidant injury to pulmonary artery endothelial cells in vitro. It is not surprising, however, that the effect of halothane on oxidant injury differs from its effect on selective agonist actions of inflammatory mediators. Our study demonstrates a differential effect of halothane on bradykinin- versus PAF-induced plasma extravasation, therefore, it is also possible that halothane could differentially affect the actions of other mediators of inflammation or injury, such as oxygen-free radicals. Interestingly, we found lower baseline plasma extravasation in halothane anesthetized animals in the current model, suggesting that halothane produces an overall inhibition of endogenous mediators of inflammation in the synovium.

In summary, we have demonstrated that halothane, but not isoflurane and pentobarbital, inhibits the ability of an important mediator, bradykinin, to stimulate a component of inflammation, plasma extravasation. Halothane’s ability to inhibit both baseline and bradykinin-induced synovial plasma extravasation may be an advantage in certain operative procedures in which postoperative edema is especially detrimental (e.g., vascular reimplantations). Additional volatile anesthetics as well as additional mediators of inflammation, such as serotonin and histamine, need to be studied to delineate further the systemic effect of halothane and possibly other volatile anesthetics on inflammation.

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References


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