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Mechanisms of the Immunological Effects of Volatile Anesthetics: A Review

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Abstract

Volatile anesthetics (VAs) have been in clinical use for a very long time. Their mechanism of action is yet to be fully delineated, but multiple ion channels have been reported as targets for VAs (canonical VA targets). It is increasingly recognized that VAs also manifest effects outside the central nervous system, including on immune cells. However, the literature related to how VAs affect the behavior of immune cells is very limited, but it is of interest that some canonical VA targets are reportedly expressed in immune cells. Here we review the current literature and describe canonical VA targets expressed in leukocytes and their known roles. In addition, we introduce adhesion molecules called $\beta 2$ integrins as non-canonical VA targets in leukocytes. Finally we propose a model for how VAs affect the function of neutrophils, macrophages and natural killer cells via concerted effects on multiple targets as examples.

1. Overview

Volatile anesthetics (VAs) have been popular drugs of choice to provide general anesthesia. These promiscuous, small molecules presumably interact with several receptors in the central nervous system (CNS) for anesthetic effect(1), and it is increasingly recognized that VAs also can target receptors outside the CNS, including those in our immune system. The studies of anesthetic effects on the immune system were already published about a century ago. Gaylord et al. reported that transplanted mammary carcinoma grew more rapidly under ether or chloroform anesthesia in mice(2), and Graham reported that ether significantly inhibited leukocyte phagocytosis of *Streptococci*(3). Although these studies were not followed up for a long time, now there is a growing interest in a potential association between the

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choice of anesthetics and patients' immunological outcomes. Our aim is to review potential VA targets on immune cells, suggest a model of VA effects on immune cells, and describe clinical implications.

2. VA targets in the immune system

2.1. Canonical VA targets

Most canonical VA targets found in the CNS are ion channels, and some are expressed on leukocytes as well. Ions clearly play critical roles in leukocytes as in neurons. Divalent cations such as Ca^{2+} and Mg^{2+} act as secondary messengers for intracellular signaling, and monovalent cations (Na^+ , K^+ , and Cl^-) regulate the membrane potential and indirectly control Ca^{2+} influx.

GABA_A receptor—GABA_A receptors are GABA-gated chloride channels and are considered important receptors underlying anesthesia(4, 5–7). They are pentamers assembled from nineteen subunits (α 1–6, β 1–3, γ 1–3, δ , ϵ etc) with a typical stoichiometry (α)₂-(β)₂- γ (or δ , ϵ). α 1–5 and β 3 are sensitive to VAs(8–15). The subunit distribution in leukocytes is in Table 1(16–19). GABA inhibits T cells proliferation and T cell-mediated delayed type hypersensitivity response(20, 21) and attenuates monocyte chemotaxis and phagocytosis(18). GABA enhances Cl^- influx, producing hyperpolarization and an attenuation of Ca^{2+} influx. We suggest that GABA acts as a negative regulator in monocytes or lymphocytes via GABA_A receptors, an action that should be further potentiated by VAs.

Glycine Receptors (GlyRs)—GlyR is a pentameric glycine-gated chloride channel, assembled from α subunits (α 1–4) and/or β subunit(22, 23). Like GABA_AR, receptors containing α 1 and α 2 are potentiated by VAs(24, 25). GlyRs are expressed in macrophages and neutrophils (Table 2)(26). Glycine enhances Cl^- influx, hyperpolarizes, attenuates Ca^{2+} influx and blunts superoxide production in stimulated neutrophils(27). Glycine attenuates the activation of LPS-treated monocytes(28) and alveolar macrophages(29). We suggest that glycine works as a negative regulator via Gly-Rs in neutrophils and monocytes/macrophages, an effect which should be potentiated by VAs.

Nicotinic Acetylcholine Receptors (nAChRs)—nAChRs are pentameric nonselective cation channels, assembled from sixteen subunits (α 1–7, α 9–10, β 2–4, γ , δ , and ϵ)(30). The α 4 β 2 receptor is expressed in macrophages and its activation enhances macrophage phagocytosis(31, 32). α 4 β 2 is also expressed in immature B cells(33). The α 7 homomer is expressed in alveolar macrophages and contributes to the reduction of pro-inflammatory cytokines upon its activation(30, 34). α 4 β 2 is inhibited by VAs, while α 7 is insensitive(35–38). We suggest that acetylcholine is a positive regulator of macrophage function via α 4 β 2, which VAs can attenuate. Although a majority of B cells mediate acquired immunity with a half-life on the order of weeks(39), some B cells have innate functions(40), which VAs may influence in unclear ways.

Serotonin receptors—The expression profile of serotonin receptors (5-HTs) in leukocytes is shown in Table 4(41, 42). Serotonin enhances macrophage phagocytosis via 5-HT_{1A} (43). 5-HT_{1B}, 5-HT_{1E} and 5-HT_{2B} induce chemotaxis of *immature* dendritic cells(41),

while 5-HT₄ and 5-HT₇ in *mature* dendritic cells reduce Th1 cytokine release. Serotonin also induces migration of mast cells and eosinophils, and is involved in T cell proliferation. In general, VAs attenuate 5-HT_{1A}, 5-HT_{2B} and 5-HT₇ activity(44). We suggest that serotonin functions as a positive regulator via 5-HTs in macrophages, dendritic cells, eosinophils, and T cells, an effect which may be attenuated by VAs.

N-methyl-D-aspartate (NMDA) receptor—The NMDA receptor is an ionotropic glutamate receptor(45). This ligand-gated nonselective cation channel is considered to be a prime VA target, assembled from NR1, NR2 (NR2A-D) and NR3(46–48). NR1 and NR2B subunits are present in resting T cells, and NR1, NR2A, NR2B, and NR2D subunits are detected in phytohemagglutinin-activated T cells(47). The inhibition of NMDA receptors impair T cell proliferation(49). Zymosan-activated neutrophils express NR1/NR2B, which may be involved in reactive oxygen species production. VAs inhibit NR1/NR2A and NR1/NR2B(50, 51). We suggest that NMDA receptors act as positive regulators in neutrophils and T cells, an effect that VAs may attenuate.

Potassium channel—Potassium channels are classified into at least four types; calcium-activated channels (Kca), voltage-gated channels (Kv), inward rectifying channels and tandem pore domain channels (K2P). Kv and Kca channels are major potassium channels in leukocytes. The K2P channel is also expressed in leukocytes and has been increasingly recognized as a prime VA target(52). Because VAs affect inward rectifying channel minimally(53), we will not review here.

Kca 3.1 and Kv1.3 are major potassium channels in T and natural killer (NK) cells(54–56), and are also expressed in macrophages(54) and neutrophils (57). VAs inhibit Kca3.1, but potentiate Kv3.1(58, 59). Among the members of K2P channel, TASK-1, -2 and -3 are expressed in T cells(59, 60), and TASK-2 is expressed in NK cells(55). Activation of K2P channel hyperpolarizes the cell and reduces activation. TASK-1, -2 and -3 are potentiated by VAs(61–65). The role of potassium channels has been studied most in T cells(59). Kca3.1, Kv1.3 and K2P channels counterbalance calcium-induced depolarization of the plasma membrane to allow more Ca²⁺ influx into T cells. The divided contribution of a K⁺ outward current in human CD3⁺ T cells is 40% through Kv1.3, 20% through Kca1.3 and 40% through K2P channels. VAs may potentiate the K⁺ efflux via their interaction with Kv1.3 and K2P channels, and reduce it via Kca3.1. Overall, the plasma membrane potential of neutrophils, macrophages and T cells, and VAs is regulated by potassium channels, an effect that VAs can alter in a heterogeneous manner.

Sodium channel—Among voltage-gated sodium channels (Nav), Nav1.2, Nav1.4, Nav1.5, Nav1.6 and Nav1.8 are sensitive to VAs(66). Nav1.5 is expressed in the late endosome of macrophage and regulates phagocytosis(67, 68). This channel is also essential for the positive selection of CD4⁺ T cells(69). We suggest that VAs attenuate the activation of macrophage phagocytic function via Nav1.5, but positive selection is a long-term process, which might not be influenced by short exposure to VAs.

2.2. Non-canonical Target: $\beta 2$ integrin

Previously, we demonstrated that 2% isoflurane exposure for 2 and 4 hours reduced neutrophil migration by 85–90% in the reverse Arthus reaction model, a well-known skin inflammation model(70). While many molecules are involved in neutrophil recruitment, the $\beta 2$ integrins are essential, as their depletion completely abolished neutrophil migration. In addition, the *ex vivo* study by Mobert et al, showing that neutrophils exposed to isoflurane or sevoflurane had reduced adhesion to human umbilical vein endothelial cells (HUVEC) provided a rationale for a closer study of the $\beta 2$ integrins(71).

Integrins are adhesion molecules consisting of α and β subunits (18 α and 8 β subunits) (Figure 1A)(72). $\beta 2$ integrins are expressed only in leukocytes, and thus are also called ‘leukocyte integrins’ and include $\alpha L\beta 2$ (aka. LFA-1), $\alpha M\beta 2$ (aka. Mac-1), $\alpha X\beta 2$ and $\alpha D\beta 2$. LFA-1 is expressed in all leukocytes and facilitates leukocyte arrest as well as immunological synapse formation on NK, T and B cells. Mac-1 is largely expressed on neutrophils, monocytes and macrophages, and underlies intravascular ‘crawling’ on the endothelium and complement-mediated phagocytosis. The *in vivo* role of $\alpha X\beta 2$ and $\alpha D\beta 2$ is still unclear. The molecules responsible for leukocyte recruitment in different tissues and pathophysiologic states are likely different(73), nevertheless, a requirement for LFA-1 and Mac-1 well described and important in leukocyte adhesion deficiency.

The major ligand binding domain (called the α I domain) for $\beta 2$ integrins is located in the α subunit(74, 75). Integrins undergo dynamic conformational changes, which include the α I domain upon activation (Figure 1A). With extracellular activation of leukocytes, LFA-1 and Mac-1 become active (inside-out signal) (Figure 1B), and then bind to their ligands. This involves the pull-down and unwinding of the $\alpha 7$ helix of the α I domain (“extended conformation”) and conformational rearrangements of the ligand binding site at the α I domain from a low- to a high-affinity configuration, only the latter of which can tightly bind to ligands (Figure 2A). The LFA-1 antagonist lovastatin works by binding to the pocket underneath this $\alpha 7$ helix, impairing ligand binding. Isoflurane and sevoflurane also bind to this ‘lovastatin site’(76–78) and inhibit LFA-1. Mac-1, which is structurally similar to LFA-1, was inhibited by isoflurane, but not by sevoflurane(76). We suggest that VAs modulate neutrophil and macrophage recruitment via LFA-1 and/or Mac-1, and phagocytosis via Mac-1, and T cell and B cell recruitment via LFA-1.

3. Integration of multiple molecular effects by VAs in leukocytes

Describing the effect of VAs on individual targets in leukocytes does not provide a complete understanding of how VAs affect their function. Bridging the gap between molecular and cellular effects requires an appreciation of how these proteins are integrated within the cell. Although additional VA targets in leukocytes may exist, it is nonetheless important to construct a model of how VAs affect the regulation of leukocyte function. VA target receptors in leukocytes are summarized in Table 6, and here we review neutrophils, macrophages and natural killer cells as examples.

Neutrophils

Circulating neutrophils are rapidly primed to extravasate and migrate towards a site of inflammation/ infection. During surgery, the timeframe of neutrophil recruitment overlaps with the exposure to VAs, making neutrophils prime targets for VA-mediated immunological effects.

Neutrophil recruitment consists of rolling, adhesion and transmigration. These events are triggered by chemoattractants and inflammatory mediators, which activate neutrophils (inside-out signal) (Figure 1B). The many molecules responsible for neutrophil recruitment, have been reviewed elsewhere in detail(73, 79). In general, activated neutrophils attach and roll along the endothelium via the selectins (E- and P- selectins on the endothelium and L-selectin on neutrophils). Then, firm adhesion on the endothelium is mediated by the $\beta 2$ integrins, principally LFA-1 and Mac-1(80). Finally neutrophils transmigrate through the endothelium to the site of inflammation/ infection to mediate a complex array of anti-bacterial effects, including phagocytosis, generation of toxic reactive oxygen species, secretion of proteases and antimicrobial peptides, neutrophil extracellular traps (NETs) as well as mediators that attract additional neutrophils, and macrophages and lymphocytes. The anti-bacterial products of neutrophils are also responsible for inflammation and tissue destruction encountered during recovery from bacterial infection, sepsis, and ischemia/ reperfusion injury (81, 82). Phagocytosis of foreign particles/ organisms is mediated by complement receptors (such as Mac-1) and Fc γ receptors(83, 84, 85).

VAs target LFA-1, Mac-1, GlyR, Kv and Kca channels, and NMDA receptors in neutrophils (Table 6). Intracellular calcium serves as a secondary messenger to activate neutrophils and its concentration is a sensitive indicator of activation(86, 87). Intracellular calcium regulates and directly activates $\beta 2$ integrins (inside-out signal)(86). NMDA receptors may enhance intracellular calcium signaling. Potassium efflux via Kv and Kca channels may counterbalance Ca²⁺ influx, sustaining the resting membrane potential and further enhancing Ca²⁺ influx as in T cells. The role of Cl⁻ efflux is more clearly understood. Resting neutrophils have an atypically high, intracellular Cl⁻ concentration (80 – 90 mM)(88). TNF- α stimulation causes Cl⁻ efflux in neutrophils. The Cl⁻ inhibitor ethacrynic acid (EA) blocks continuous Cl⁻ efflux, cell spreading and superoxide production in TNF- α -stimulated neutrophils. Furthermore, resuspension of neutrophils in Cl⁻ free medium results in massive Cl⁻ efflux, Ca²⁺ influx, enhanced adhesion and superoxide production, demonstrating that Cl⁻ efflux and Ca²⁺ influx themselves lead to neutrophil and $\beta 2$ integrin activation(89). GlyR, on the other hand, enhances Cl⁻ influx. Cl⁻ influx hyperpolarizes the cell membrane, which presumably reduces Ca²⁺ influx(27) and the likelihood of neutrophil activation. Figure 2 summarizes the potential effects of VAs on neutrophils.

Macrophages—Macrophages are considered a “professional” phagocyte. Monocytes circulate through bone marrow and extravasate to tissues and differentiate into macrophages (90). Macrophages can be divided into different subsets based on their molecular expression profiles; “classically” activated macrophages are M1 macrophages and “alternatively” activated macrophages are M2 macrophages. M1 macrophages can be induced by IFN- γ and LPS stimulation *in vitro* and they mediate host defense with high microbicidal activity, while

M2 macrophages are induced by IL-4 and IL-13 *in vitro* and mediate anti-inflammatory effect, phagocytosis and help tissue healing (91).

The GlyR, GABA_A-receptor, nAChR, 5-HT_{1A}, LFA-1, Mac-1, Kca3.1, Kv1.3, and Nav1.5 are all VA targets on macrophages / monocytes. GlyR allows Cl⁻ influx and hyperpolarizes the membrane, inhibiting a rise in intracellular calcium, superoxide and TNF- α secretion(26, 29). Activation of the GABA_A reduced IL-6 and IL-12 production in LPS stimulated macrophages(92), whereas stimulation of nAChR $\alpha 4\beta 2$ with agonists attenuated intracellular calcium and NF-kB activation(32), but enhanced phagocytosis(31). Both Kca3.1 and Kv1.3 regulate calcium influx(93), and their blockade reduced chemotactic migration (94, 95). Nav1.5 is expressed on the late endosome and contributes to the generation of prolonged and localized calcium oscillations during phagocytosome formation(67). The activation of 5-HT_{1A} enhances phagocytosis(96). The interactions among these ion channels and receptors have not been much studied in macrophages, but the final effect is unlikely to be a simple sum of the individual actions. We summarized the interactions of VAs on target receptors in macrophages in Figure 3.

NK cells—NK cells are a phenotypically distinct population of cytotoxic lymphocytes that are critical for innate immunity. They express a number of activating and inhibitory receptors, and can respond to a variety of target cells, including viral infected cells and tumor cells, and lyse them without prior immunization (97). The decision to lyse target cells is determined by a delicately balanced input to NK cells from these activating and inhibitory receptors. LFA-1 is one of critical activating receptors on NK cells involved in binding to target cells (conjugation) and providing signals to lyse target cells (98). The binding of LFA-1 to ICAM-1 reorganizes cytoskeletal structures within NK cells and causes degranulation and cell lysis(99). An elevation of intracellular calcium is a requisite step for this killing process(100). Potassium channels counterbalance the depolarization induced by a rise of intracellular calcium level(55), and have been shown to be involved in the adhesion of NK cells to target cells as well as cytotoxicity(101). LFA-1, Kca3.1, Kv3.1 and TASK2 are all receptors that VAs can target in NK cells. Among them, LFA-1 and TASK2 are both involved in LFA-1 dependent adhesion to target cells (55). A TASK 2 antagonist attenuated calcium influx, preventing depolarization and the release of lytic granules. Similarly, blockade of Kv1.3 reduced calcium influx(55), and thereby NK cell degranulation(56). On the other hand, Kca1.3 blockade enhanced the NK degranulation, but did not affect attachment to target cells(56). The summary is in Figure 4, demonstrating the various potential effects of VAs on NK cell functions

Clinical considerations in the perioperative periods

Our body has multi-layered defense mechanisms against the outside world. Skin and mucosa are the most exposed tissues and acts as the first line of defense against various organisms. Surgical procedures usually disrupt these barriers and introduce various organisms to otherwise sterile sites. Also tissue injury itself triggers acute inflammation ('sterile inflammation'). Adequate recruitment of circulating leukocytes to the site is critical for cleaning the site and promoting wound healing. In addition, some surgical procedures involve more extreme forms of pathophysiology such as ischemia-reperfusion. During tumor

resection, immune cells may have to keep fighting to eradicate disseminated tumor cells. The role of VAs in these circumstances is briefly reviewed.

Ischemia- Reperfusion injury

Ischemia- reperfusion is a common experience in many surgical procedures (ex. Pringle maneuver, unclamping of cross-clamped arteries, reestablishment of blood flow during transplantation) or after relief of vascular obstruction (ex. relieving malrotation of guts, embolectomy). This is considered an extreme example of sterile inflammation. Among immune cells, neutrophil recruitment plays a central role in the pathogenesis (102, 103), and the activation of $\beta 2$ integrins is critical. The benefit of VAs in ischemia-reperfusion has been shown in various organs and tissues including kidney(104), lung(105) and coronary artery/ myocardium in animal models(103, 106). In a study using healthy human volunteers, sevoflurane (even at 0.5-1%) protected forearm ischemia-reperfusion injury via an attenuation of neutrophil activation(107). The benefit of VAs in clinical settings is also reported in cardiac surgery (108). These results, while sparse, support a role for VAs in mitigating sterile inflammation.

Tumor immunology

Although surgical resection is considered a gold standard of solid tumor management, recurrence or metastasis remains the major cause of death (109). The postulated, underlying mechanisms include the mechanical dissemination of tumor cells intraoperatively, and promotion of local and distal metastasis by attenuation of cell-mediated immunity(110). A growing literature suggests that the type of anesthesia/anesthetics is associated with the frequency of tumor recurrence or metastasis. In general less general anesthesia is associated with less recurrence or metastasis (111–115), and these studies were previously reviewed in detail (116). A recent retrospective study showed that patients receiving VA for their surgery were associated with a hazard ratio of 1.59 for death on the univariate analysis and 1.46 after the multivariate analysis, as compared to the patients receiving total intravenous anesthesia, suggesting that Vas may not be beneficial in tumor surgery (117). Perioperative NK cell suppression correlates with higher recurrence and mortality in patients (118, 119). As stated above, VAs should impair NK conjugation and degranulation via inhibiting LFA-1 and TASK2. On the other hand, VAs could enhance degranulation through their interaction with Kca3.1 and Kv1.3. Given that conjugation precedes degranulation, VAs presumably impair NK cell cytotoxicity, but further study is warranted to confirm these provocative findings.

Infection

Perioperative infection is one of the most common perioperative complications. Von Dossow et al. examined alcoholic patients who underwent resection of upper gastrointestinal tract under general anesthesia either with propofol or isoflurane. Postoperative infection rate was lower in the propofol group (23%) than in the isoflurane group (67%)(120). Chang et al. studied the incidence of surgical site infection (SSI) in patients who underwent orthopedic procedures under regional anesthesia (spinal or epidural anesthesia) or general anesthesia and found that the general anesthesia arm had a higher incidence of SSI (2.8%) over the regional anesthesia arm (1.2%)(121). Although the detailed information of the general

anesthetics was not reported, VAs would presumably be the major anesthetics in their general anesthesia group. Leukocyte recruitment and subsequent killing of microbes are required to minimize perioperative infection. Several animal studies have demonstrated that VAs modify leukocyte adhesion and recruitment(71, 106, 122, 123, 124, 125, 126, 127). The number of recruited leukocytes to tissues is determined by a balance between pro-inflammatory signals and anti-inflammatory signals (resolution). The intravital microscopic experiment of mesenteric circulation in LPS-induced inflammation showed that isoflurane exposure increased the speed of leukocyte rolling(123), which could lead to impaired adhesion and transmigration. Our study using the reverse Arthus reaction model in mice showed impaired neutrophil migration by isoflurane(128). While the proinflammatory mediators in these models recruit leukocytes, resolution agonists such as lipoxins and resolvins limit further recruitment(129), but the effect of VAs on resolution agonists was not reported(127). The role of VAs on bacterial killing has also been studied. *Ex vivo* studies in patients (ASA I-III) undergoing abdominal surgery showed a reduction of granulocyte phagocytosis and oxidative burst after one hour of sevoflurane or xenon anesthesia(130). Also in an *ex vivo* study using patients who underwent orthopedic procedures, a time-dependent reduction of phagocytic function and bacterial killing was reported to occur under isoflurane anesthesia(131).

Postoperative cognitive dysfunction (POCD)

Change in personality or cognitive ability following surgery is considered a significant form of morbidity. The causes are likely multifactorial, including a host of preoperative comorbidities, and inflammation, stress and infection resulting from surgery and anesthesia. TNF- α and its downstream mediator IL-1 β play a role in cognitive dysfunction in animal models(132, 133). Cognitive dysfunction produced by TNF- α / IL-1 β in mice occurred via the α 5GABA_A receptor. Activation of this receptor appears to impair memory and IL-1 β induced prolonged α 5 GABA_A receptor expression(134). Furthermore, isoflurane caused a persistent tonic current via α 5GABA_A receptor(135).

Microglia, which are analogous to macrophages in the brain, secrete a variety of proinflammatory mediators, so attenuating this neuroinflammation may help to reduce post-operative memory and perhaps cognitive deficits. In a study of isolated microglia, isoflurane had little effect on LPS-stimulated proinflammatory cytokine release, while sevoflurane enhanced it (136). In patients undergoing esophageal tumor resection under sevoflurane anesthesia or propofol anesthesia, cognitive decline was more apparent in the sevoflurane anesthesia group, and was associated with higher plasma IL-6 and TNF- α concentrations. Finally, in a rare randomized controlled study of older patients getting spinal surgery, and with mild pre-existing cognitive deficits, those receiving sevoflurane anesthesia declined more rapidly as compared to regional or propofol(137). These studies suggest that VAs may contribute to post-operative cognitive decline, but it is becoming clear that many other factors are involved.

4. Summary

Although clinical studies on the association between VAs and immunological issues are limited, this review highlights a molecular biological basis of VA-induced immunomodulation and hopefully facilitates clinical studies on perioperative immunological outcomes in the future.

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Figure 1. The conformational changes of $\beta 2$ integrins with inside-out signal

(A) With activation, the conformation of $\beta 2$ integrins changes from a bent conformation (left) to an extended conformation (right). $\beta 2$ integrins can bind to their ligands only when they are fully activated. α ; α subunit, β ; β subunit. The blue arrow shows the downward displacement of C terminus in the α I domain. (B) The scheme of inside-out signal, $\beta 2$ activation and subsequent ligand binding.

The effect of volatile anesthetics on target receptors

- a) $\beta 2$ integrin activation-- reduce
- b) Intracellular calcium influx
 - NMDA-R: reduce
 - Gly-R: reduce by potentiating Cl^- influx
 - Kca: reduce by inhibiting K^+ efflux (?)
 - Kv: enhance by potentiating K^+ efflux (?)

Figure 2. The concerted effects of volatile anesthetics on target receptors in neutrophils
 Signals via cytokine and/or chemokine receptors increase intracellular calcium concentration and activate $\beta 2$ integrins. Intracellular calcium influx can be enhanced by the N-methyl-D-aspartate (NMDA) receptor, voltage-gated potassium channel (Kv) and calcium-activated potassium channel (Kca), and attenuated by the glycine receptor (Gly-R). The effect of volatile anesthetics on these targets is summarized. The subtypes of Kv and Kca channels are not known and need to be studied as well as the effect of volatile anesthetics.

Figure 3. The targets of volatile anesthetics in macrophages and their biological roles
 VA target receptors and ion channels in macrophages are shown. ER =endoplasmic reticulum.

Figure 4. The targets of volatile anesthetics on natural killer cells and their biological roles
 Once natural killer (NK) cells bind to target cells (conjugation), they polarize and degranulate granzyme, perforin and FAS ligand to produce target cell cytotoxicity. LFA-1 is critical for adhesion to target cells (conjugation), and TASK2 is also involved in LFA-1 dependent adhesion as well as degranulation. Kca3.1 and Kv1.3 channels are involved in degranulation.

Table 1Expression profiles of GABA_A receptor subunits in human /rodent leukocytes

	Human	Rodent
Neutrophil		
Monocyte/macrophage	<u>α1</u> , β2	<u>α1</u> , <u>α2</u> , <u>β3</u> , δ
Dendritic cell		
Eosinophil		
Mast cell		
NK cell		
T cell	<u>α1</u> , <u>α3</u> , α6, β2, <u>β3</u> , γ2, δ	<u>α1</u> , <u>α2</u> , <u>α3</u> , <u>α4</u> , <u>α5</u> , α6, <u>β3</u> , γ1, γ3, δ
B cell	<u>α1</u> , <u>α3</u> , β2	
PBMC	<u>α1</u> , <u>α3</u> , <u>α4</u> , β2, β3, γ2, δ	

The underlines demonstrate the subtypes that are strongly affected by VA. PBMC can be monocyte, macrophage, T cell, B cell, or NK cell.

Table 2

Expression profiles of glycine receptor subunits in rodent leukocytes

Neutrophil	<u>α2</u> , β
Monocyte/macrophage	<u>α1</u> (Kupper cell), <u>α2</u> (splenic, alveolar), β
Dendritic cell	
Eosinophil	
Mast cell	
NK cell	
T cell	
B cell	

The underlines demonstrate the subtypes that are strongly affected by VA.

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Table 3

Expression profiles of nAChRs in rodent leukocytes

Neutrophil	$\alpha 7$ (alveolar)
Monocyte/ macrophage	<u>$\alpha 4\beta 2$</u> , $\alpha 7$
Dendritic cell	
Eosinophil	
Mast cell	
NK cell	
T cell	
B cell	<u>$\alpha 4\beta 2$</u> (pre/pro-B cell lineage)

The underlines demonstrate the subtypes that are strongly affected by VA.

Table 4

Expression profiles of 5-HT subtypes in human and rodent leukocytes

	Human	Rodent
Neutrophil		<u>5-HT_{1A}</u> , 5-HT _{2A}
Monocyte/ macrophage		
Dendritic cell	5-HT _{1B} , 5-HT _{1E} , 5-HT _{2A} , <u>5-HT_{2B}</u> , 5-HT ₃ , 5-HT ₄ , <u>5-HT₇</u>	
Eosinophil	5-HT _{2A}	5-HT _{2A}
Mast cell	<u>5-HT_{1A}</u>	<u>5-HT_{1A}</u>
NK cell		
T cell	5-HT _{1B} , 5-HT _{2C}	5-HT _{1B} , 5-HT _{2A} , <u>5-HT₇</u>
B cell	<u>5-HT₃</u>	

* The underlines demonstrate the subtypes that are strongly affected by VA.

Table 5

Expression profiles of NMDA receptor subunits in human and rodent leukocytes

	Human	Rodent
Neutrophil		<u>NR1, NR2B</u>
Monocyte/ macrophage		
Dendritic cell		
Eosinophil		
Mast cell		
NK cell		
T cell	<u>NR1, NR2A, NR2B, NR2D</u>	<u>NR1</u>
B cell		

*
The underlines demonstrated the subtypes that are affected by VAs.

Table 6

Possible VA target receptors on leukocytes

Neutrophil: Gly-R, NMDA-R (?), LFA-1, Mac-1, Kv and Kca channels
Macrophage/ Monocyte: Gly-R, GABA _A -R, nAChR ($\alpha 4\beta 2$), 5-HT _{1A} , LFA-1, Mac-1, Kca3.1, Kv1.3, Nav1.5
NK cell: LFA-1, TASK2 (K2P channel), Kca3.1, Kv1.3
T cell: GABA _A -R, 5-HT ₇ , AMPA-R, NMDA-R, LFA-1, K2P, Kca3.1, Kv1.3
B cell: GABA _A -R, nAChR, 5-HT ₃ , Kainate receptor, K2P channel, LFA-1