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New insights in the systemic and molecular underpinnings of general anesthetic actions mediated by GABA_A receptors

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Abstract

Purpose of the review—This review highlights novel insights into the role of GABA_A receptors in mediating clinically relevant actions of anesthetic agents.

Recent findings—GABA_A receptors in the hippocampus are located on glutamatergic pyramidal cells and GABAergic interneurons. Etomidate-induced inhibition of a synaptic correlate of learning and memory is caused by receptors on non-pyramidal neurons, likely on interneurons that incorporate $\alpha 5$ -subunits. Selective enhancement of $\alpha 2$ -subunit containing GABA_A receptors in the spinal cord provides antihyperalgesia against inflammatory and neuropathic pain without causing sedation, motor impairment and tolerance development. Inflammation, traumatic brain injury and exposure to anesthetic agents modify the expression patterns of GABA_A receptors in a subtype-specific manner. These modifications may persist for weeks. The neuroactive steroid alphaxalone causes fast-onset and short duration anesthesia in humans. Cardiovascular and respiratory side effects are less severe than with propofol.

Summary—Identification of the molecular and cellular substrates involved in anesthesia and insights into disease- and drug-induced alterations in the expression patterns of GABA_A receptors in the central nervous system are emphasizing the need for individualized anesthesia care. Introducing neuroactive steroids into clinical anesthesia is expected to reduce cardiovascular and respiratory side effects.

Keywords

$\alpha 2$ - GABA_A receptors; $\alpha 5$ - GABA_A receptors; amnesia; antihyperalgesia; inflammation; brain injury

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Introduction

GABA_A receptors are ligand-gated chloride channels composed of five protein subunits.[1] Their endogenous agonist γ -aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). GABA_A receptors are targeted by most anesthetic agents in current clinical use.[2] Acting as positive allosteric modulators they enhance the function of GABA_A receptors, thereby decreasing the activity and excitability of central neurons. Nineteen different protein subunits of the GABA_A receptor have been identified, giving rise to many receptor subtypes fulfilling different physiological functions (Figure 1).[3] These subtypes are not uniformly distributed throughout the CNS. Even within the same brain region their expression varies between cell types and even subcellular regions.[4] It is generally accepted that at clinically relevant concentrations anesthetic agents such as propofol, etomidate and isoflurane, interact with several distinct GABA_A receptor subtypes.[5] The specific contributions of GABA_A receptor subtypes to the pharmacological properties of general anesthetic drugs are currently under investigation. Here we will highlight novel findings of how anesthetics interfere with learning and memory and how they affect spinal pain processing. We will review recent evidence that anesthetic agents, inflammation and brain injury can cause rapid and long-lasting changes in the surface expression of GABA_A receptors, which in turn alter the patient's sensitivity to anesthetic drugs. Because GABA_A receptors play a pivotal role in regulating neuronal excitability, it is not surprising that their activity is modulated by endogenous molecules. For example, endogenous steroids are known to act as powerful positive allosteric modulators at GABA_A receptors.[6] We will also discuss efforts to re-introduce alphaxalone into clinical anesthesia and elucidate the potential benefits of neuroactive steroids.

Impairment of learning and memory by anesthetic agents

The hippocampal formation takes a central role in learning and memory. Anesthetic actions within the hippocampus itself are thought to cause anesthetic-induced amnesia.[7] This idea is supported by the observation that at small amnestic concentrations anesthetic agents depress hippocampal long term potentiation (LTP), a major cellular mechanism that underlies learning and memory.[8,9] It has been discovered previously by studying α 5-global knockout mice that etomidate impairs synaptic plasticity and memory via modulation of α 5-subunit containing GABA_A receptors. [9] which are expressed primarily in the hippocampus[10,11] and that this impairment can be reversed by the negative allosteric α 5-selective modulator, L-655,708.[12] However, the neuronal population at which etomidate effects these changes is still unknown. It was generally assumed that pyramidal neurons, e.g. in CA1, but also other hippocampal regions, would be such cellular targets, as these neurons have been shown to play a role in learning and memory, and α 5-GABA_A receptors on these neurons would inhibit these neurons via producing an inhibitory tonic current. However, in conditional knockout mice in which the α 5-subunit is lacking selectively in CA1 pyramidal neurons, etomidate surprisingly suppressed long-term potentiation in CA1 pyramidal neurons.[13] The level of tonic current was similar to levels in floxed control mice and α 5 global knockout mice. In the CA1-specific α 5 knockout mice, in the presence of etomidate there was a non-significant trend to an increase in tonic current, to levels not different from

those in the global $\alpha 5$ -knockout mice. This indicates that etomidate's effect on tonic inhibition in CA1 pyramidal neurons is not the mechanism by which etomidate inhibits learning and memory. Furthermore these findings suggest that $\alpha 5$ -GABA_A receptors on non-pyramidal cells, potentially interneurons, may be the targets for the amnestic action of etomidate.

To determine whether modulation of $\beta 3$ -containing GABA_A receptors by etomidate impairs learning and memory, etomidate was tested in $\beta 3$ (N265M) mice in which the $\beta 3$ -subunits have been rendered insensitive to modulation by etomidate.[14] In hippocampal slices of these mice, etomidate did not enhance tonic inhibition, but it still blocked long-term potentiation in CA1 pyramidal cells, and it impaired fear conditioning to context.[15] These results suggest a dissociation between tonic inhibition and impairment of memory: while $\beta 3$ -GABA_A receptors mediate enhancement of tonic inhibition, they do not mediate impairment of memory elicited by this drug. In conjunction with the observation that amnestic concentrations of etomidate enhance receptor activity at recombinant $\alpha 5\beta 2\gamma 2$ - and $\alpha 5\beta 3\gamma 2$ -, but not $\alpha 5\beta 1\gamma 2$ -GABA_A receptors,[15] the findings mean that etomidate most likely impairs memory via receptors containing the $\beta 2$ -subunit, although they do not rule out the possibility that etomidate may inhibit CA1 pyramidal neurons by mechanism different from tonic inhibition, e.g., slow phasic inhibition. Performing similar experiments with $\beta 2$ (N265M)-mice would unequivocally confirm the role of $\beta 2$ -GABA_A receptors in LTP suppression and fear conditioning to context.

Actions of benzodiazepines in the spinal cord

Systemically applied classical benzodiazepines do not have an analgesic action. However, it is known that in rodents intrathecally applied and thus primarily spinally acting benzodiazepines have an antihyperalgesic action in a variety of pain models, including models of inflammatory and of neuropathic pain.[16] This antihyperalgesic action does not involve supraspinal sites.[17] In a recent study, antihyperalgesic effects and potential side effects (such as sedation, muscle relaxation and motor impairment) of positive allosteric modulation of single GABA_A receptor subtypes as defined by their α -subunits was examined in a panel of mice in which three out of the four diazepam-sensitive α -subunits $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ were rendered diazepam-insensitive by histidine to arginine knock-in point mutations.[18] Modulation of $\alpha 2$ -containing GABA_A receptors elicited approximately 60% of the maximally possible anti-hyperalgesic effect (with other contributions provided by $\alpha 3$ -GABA_A receptors and $\alpha 5$ -GABA_A receptors). While positive modulation of $\alpha 1$ -GABA_A receptors elicits sedation, positive modulation of $\alpha 2$ -GABA_A receptors and of $\alpha 3$ -GABA_A receptors elicits muscle relaxation, while modulation of $\alpha 3$ -GABA_A receptors but not of $\alpha 2$ -GABA_A receptors impairs motor coordination.

In mice in which $\alpha 2/\alpha 3/\alpha 5$ -GABA_A receptors are sensitive to diazepam, with an acute dose of diazepam, maximum possible anti-hyperalgesia is achieved, but after 10 days of chronic administration of diazepam, this effect is completely abolished, indicating development of tolerance. However, in mice in which only $\alpha 2$ -GABA_A receptors are modulated by diazepam, both acute and a 10 day chronic administration of diazepam result in approximately 60% of the maximal anti-hyperalgesic action. Thus, there is no development

of tolerance. Development of tolerance may be circuit-specific as these mice develop tolerance to the locomotor increasing effect of diazepam in these mice. It is currently not known whether in these mice tolerance develops to the muscle relaxant action of diazepam. Overall, $\alpha 2$ -specific modulation would likely have strong antihyperalgesic actions but relatively limited unwanted side effects. It could further be demonstrated that in animals in which diazepam or midazolam only modulate $\alpha 2$ -GABA_A receptors the antihyperalgesic action of diazepam or midazolam occur at doses that already elicit sedative actions in mice in which these compounds only modulate $\alpha 1$ -GABA_A receptors.

Drugs acting only at a single subtype of benzodiazepine-sensitive GABA_A receptors remain to be introduced into clinical practice in the future. However, the study of Ralvenius and coworkers suggests variable potency and efficacy of different benzodiazepines in modulating $\alpha 1$ -GABA_A receptors.[18] Thus, at slightly sedating concentrations some benzodiazepines approved for human use may display anti-hyperalgesic actions. When clonazepam and clobazam were tested at mildly sedating equiconvulsive dose in cutaneous UVB irradiation-induced secondary hyperalgesia in humans, these compounds accelerated recovery from hyperalgesia by 28.6% (CI 4.5–52.6) and 15.7% [CI 0.8–30.5], respectively.[19] These results provide the first proof-of-principle evidence supporting an antihyperalgesic action of GABA_A receptor modulation in humans.

Anesthetic agents alter surface expression of GABA_A receptors

We have mentioned previously that $\alpha 5$ -GABA_A receptors mediate the amnesic action of etomidate.[9] A new study found that etomidate exerts cellular changes and pharmacological actions long after administration of the drug.[20] A single dose of etomidate (8 mg/kg) impairs memory performance in the novel object recognition task both 24 hours and 72 hours, but not 1 week after drug administration. Likewise, field postsynaptic potential slope was decreased by etomidate at the 24 hour time point, but not at the 1 week timepoint. In hippocampal slices, the $\alpha 5$ -GABA_A receptor mediated current density was increased in CA1 pyramidal neurons 24 hours, 72 hours, and 1 week after administration of etomidate, but not 2 weeks after administration of etomidate. This indicates that $\alpha 5$ -GABA_A receptor function is altered long after etomidate is presumably eliminated from the organism. The surface expression of $\alpha 5$ -GABA_A receptors but not total expression of $\alpha 5$ -GABA_A receptors was increased 24 hours and 1 week, but not 2 weeks after administration of etomidate, suggesting an intracellular redistribution of these receptors. The nature of these sustained changes is currently unknown. It is noteworthy that in neuronal cultures treated with etomidate-incubated astrocytes tonic current density is increased, compared to vehicle-treated astrocytes.[20] This may suggest that GABA_A receptors on astrocytes may contribute to enhancement of tonic current in CA1 pyramidal neurons. Negative allosteric modulation of $\alpha 5$ -GABA_A receptors reversed the memory deficits. Furthermore, the authors tested the effects of the volatile anesthetic isoflurane on cell-surface expression of $\alpha 5$ -GABA_A receptors. At a low, sedative dose isoflurane (0.7%, 20 minutes) was ineffective. However, at a higher, anesthetizing dose (1.3%, 60 minutes) isoflurane significantly increased cell-surface expression of $\alpha 5$ -subunits, leaving the expression of δ -subunits unchanged. [20]

In another study the effect of propofol on the cell-surface expression of $\beta 3$ -GABA_A receptor subunits were explored in cultured cortical neurons and in mice.[21] Propofol (10 μ M, up to 60 min) facilitated membrane accumulation of $\beta 3$ -subunits and, unlike etomidate, increased the amplitudes of miniature IPSCs. These findings indicate that propofol potentiates synaptic inhibition via increasing the density of postsynaptic GABA_A receptors. Furthermore, propofol blocked the interaction between $\beta 3$ -subunits and the β -adaplin subunit of adaptor protein 2, thereby reducing endocytosis of GABA_A receptors.[21] Similar to propofol, diazepam can rapidly strengthen GABA_A receptor-mediated synaptic transmission via increasing the number of postsynaptic receptors.[22] Interestingly, this effect required sustained neuronal activity and was not apparent under resting conditions. However, it is important to note that the duration of drug exposure seems to have a major impact on the changes induced in GABA_A receptor trafficking. When cultured hippocampal neurons were treated with flurazepam for 24 hours (in the aforementioned studies propofol and diazepam were applied for up to one hour only), a dramatic decrease in surface and total levels of $\alpha 2$ -subunit containing GABA_A receptors occurred, whereas levels of $\alpha 1$ -subunits remained unaltered.[23]

In summary, agents acting as positive allosteric modulators at GABA_A receptors alter the gating kinetics and in addition can rapidly alter receptor trafficking. Although the underlying molecular mechanisms remain to be studied, there is evidence that these effects are specific for different GABA_A receptor subtypes and strongly depend on the duration of drug exposure. Furthermore, different anesthetic agents seem to modify the surface expression of GABA_A receptors in different ways.

Expression of GABA_A receptors is modified by inflammation and brain injury

Systemic inflammation can cause cerebral dysfunction.[24–26] It has been proposed that elevated levels of the proinflammatory cytokine interleukin-1 β compromise memory and learning by increasing the surface expression of $\alpha 5$ -GABA_A receptors in hippocampal pyramidal cells.[27] This raises the question whether inflammation modifies neuronal sensitivity to general anesthetic agents by altering the expression patterns of GABA_A receptors in the brain. Pretreatment with the bacterial endotoxin lipopolysaccharide enhanced the hypnotic effects of etomidate and isoflurane in mice.[28] Furthermore, lipopolysaccharide and etomidate impaired contextual fear memory. In cultured hippocampal and cortical neurons interleukin-1 β enhanced agonist-induced currents across GABA_A receptors and promoted the actions of etomidate at the same receptors.[28] Collectively these findings suggest that systemic inflammation increases the surface expression of GABA_A receptors thereby inducing neuronal sensitivity to general anesthetics.

Traumatic brain injury (TBI) is recognized as a major health problem.[29] After initial brain damage secondary pathologies develop over the following days. Anesthetic agents acting via GABA_A receptors are frequently used in these patients' anesthesia to induce and maintain general anesthesia.[30] Drexler and coworkers assessed the expression of GABA_A receptor subunit mRNAs in rats 6 hours and 4 months post-TBI in the hippocampus and thalamus.

[31] In the hippocampus the expression of $\alpha 1$ -, $\alpha 2$ -, $\alpha 5$ -, $\beta 2$ -, $\beta 3$ - $\gamma 2$ - and δ - GABA_A receptor subunit mRNAs were down-regulated 6 and 24 hours post-TBI. However, these changes were transient and disappeared after 4 months. In the thalamus $\alpha 1$ -, $\alpha 4$ -, $\alpha 2$ -, $\beta 2$ -, $\gamma 2$ - and δ - GABA_A receptor subunit mRNAs were downregulated by TBI but recovery was largely absent for some of these subunits. How these dramatic changes relate to the surface expression of GABA_A receptors and how the patient's sensitivity to general anesthetic agents after TBI is altered remains to be investigated.

Neurosteroid analogues for providing anesthesia

Althesin® is a short acting intravenous anesthetic agent that was used in clinical anesthetic practice from 1972 to 1984. It is a mixture of the neuroactive steroids alphaxalone and alphadolone solved in water with the aid of the formulation vehicle Cremophor EL® and was withdrawn from the market because of hypersensitivity reactions caused by the excipient.[32] Synthetic neurosteroids like alphaxalone are analogues of naturally occurring neurosteroids.[33] Therefore these agents are expected to have fewer cardiovascular and respiratory side effects and the development of tolerance is reduced. Stress and brain injury both enhance endogenous neurosteroid de novo synthesis, which is probably part of a self-protecting reaction chain.[34] Moreover, a large body of evidence has accumulated that neurosteroids provide neuroprotection.[35] When administered together, neurosteroids and anesthetic agents can enhance GABA_A receptor-mediated inhibition in a synergistic manner.[36] This finding suggests that the combined use of anesthetics and neurosteroids may significantly reduce dose requirements needed for clinical anesthesia, which in turn should reduce cardiovascular and respiratory side effects.

As mentioned before, the use of Althesin® in clinical anesthesia was discontinued due to the hypersensitivity reactions caused by Cremophor EL®. Therefore Goodchild and coworkers replaced Cremophor EL® with 7-sulfobutyl-ether- β -cyclodextrin (betadex), which has been successfully used to dissolve hydrophobic drugs in water for intravenous injections in humans.[37] Alphaxalone induced fast-onset anesthesia in rats at the same dose for both formulations. Remarkably, the dose that caused death in 50% of animals (LD₅₀) was more than twofold higher with betadex as the formulation vehicle. A phase 1c trial compared the efficacy and safety of Phaxan™, an aqueous solution of alphaxalone and betadex with propofol.[38] Similar to propofol, Phaxan™ caused fast-onset and short duration anesthesia. However, respiratory depression and the decrease in systolic and diastolic blood pressure were less pronounced. Furthermore subjects did not complain of pain on injection with Phaxan™.

Conclusions

The sedative, amnestic, anxiolytic, hypnotic, muscle relaxant and antihyperalgesic actions of anesthetic agents involve distinct sub-populations of GABA_A receptors. This observation is raising the question if there are ways to achieve some of these actions but not others by making use of subtype-selective drugs. Proof of principle studies have shown that amnesia can be achieved or prevented by selective modulation of receptors containing $\alpha 5$ -subunits.[9,12,39] Furthermore, drugs that enhance the function of $\alpha 2$ - but not $\alpha 1$ -subunits should be

useful for providing antihyperalgesia against inflammatory and neuropathic pain.[18] It is also important to consider that the expression pattern of GABA_A receptor subtypes in the brain and spinal cord is altered by therapeutically relevant factors including drug usage[40], inflammation[41], injury[31], disease[42,43], age[44,45] and pregnancy (Figure 2)[46]. These novel findings are strengthening the need for individualized anesthesia care. Finally the use of neuroactive steroids is expected to reduce cardiovascular and respiratory side effects of anesthetic agents in current use.

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Summary points

1. Selective enhancement of $\alpha 2$ -GABA_A receptor function causes antihyperalgesia without sedation.
2. The brain expression pattern of GABA_A receptor subunits is substantially altered by the administration of anesthetic drugs, inflammation and brain injury, which in turn modifies the patient's sensitivity to anesthetic agents.
3. Cardiovascular and respiratory side effects in human subjects are less severe if anesthesia is induced with alphaxalone than with propofol.

Figure 1. GABA_A receptors and anesthetic actions

GABA_A receptors assemble from five protein subunits.[47] Most GABA_A receptors are composed of two α -, two β - and a γ -subunit. However, nineteen different protein subunits are expressed in central neurons, giving rise to many potentially existing receptor subtypes. Some of these are indicated on the upper right. There is evidence that receptor subtypes harboring $\alpha 1$ - and $\beta 2$ subunits mediate the sedative actions of benzodiazepines, etomidate and propofol.[48,49] Furthermore, GABA_A receptors incorporating $\alpha 2$ -subunits mediate the anxiolytic and antihyperalgesic actions of benzodiazepines.[18,50] $\alpha 5$ -containing GABA_A receptors are densely expressed in the hippocampus and mediate the amnestic effect of anesthetics.[9] The amnestic, sedative, anxiolytic and hypnotic properties of anesthetic agents are mediated by GABA_A receptors in the brain whereas antihyperalgesia and immobility is caused by spinal receptors.[2]

Figure 2.

Factors affecting the expression pattern of GABA_A receptor subunits, potentially altering the patient's sensitivity to anesthetic drugs.