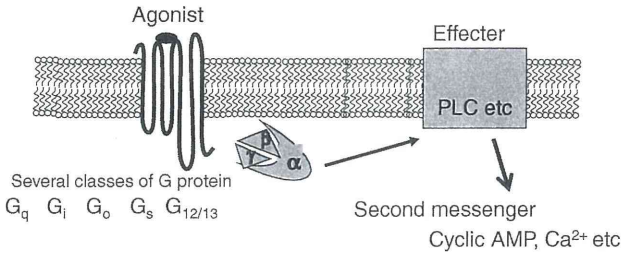


Fig. 1 Intracellular signaling of G protein coupled receptor

Table 1 Signaling of G protein coupled receptor

G protein	G_q	$G_{i/o}$	G_s
Effecter	PLC↑	Adenylate cyclase↓	Adenylate cyclase↑
Second messenger	IP_3 ↑ DAG↑	cAMP↓	cAMP↑
Intracellar reaction	PKC↑ Ca^{2+}↑	PKA↓	PKA↑
Receptor	M_1	M_2	β-Adrenergic
	M_3	μ Opioid	
	Substance P		
	Orexin 1		
	$5HT_{2A}$		
	mGluR1		
	mGluR1		

Table 2 The effects of volatile anesthetics on Gq coupled receptor function

	Halothane	Isoflurane	Enflurane	Desflurane	Sevoflurane
M_1	↓	→		↓↑	↓
M_3	↓	↓		→	↓
$5HT_{2A}$	↓				
$5HT_{2C}$		↓	↓		
mGluR1	→				
mGluR5	↓				
Substance P	↓	↓	↓		↓
Orexin 1	↓	↓	↓		

reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. This system has been well characterized, and has proven useful for studying the effects of anesthetics and analgesics on G_q coupled receptors.

Muscarinic acetylcholine receptors

In G_q coupled receptors, muscarinic acetylcholine receptors (MRs) have been paid much attention to as the target of the anesthetics and analgesics. This is because MRs are involved in various neuronal functions in the central nervous system (CNS) and the autonomic nervous system [10]. Cholinergic antagonism interferes with learning behavior, whereas cholinesterase inhibitors enhance learning [11]. Furthermore, the inhibition of MRs lead to sedation or non-rapid eye movement sleep [12]. The therapeutic potential of muscarinic antagonists is compromised by several effects on the autonomic nervous system, including dry mouth, tachycardia, constipation, urinary retention, and pupillary dilation [13]. Recent molecular cloning studies have revealed the existence of five subtypes of MRs (M1R-M5R) [14, 15]. Using pharmacological techniques, many of the muscarinic responses in peripheral tissues have been studied thoroughly. However, relatively little is known about the functional roles of individual subtypes of MRs in the CNS. Recent studies of their anatomic distribution have been used to predict their functions in the CNS. For example, cortical and hippocampal M1R are involved in memory and learning [16].

To date, several investigators have studied the effects of anesthetics on MRs. Anthony et al. [17] reported that chloroform, enflurane, isoflurane and halothane increased the affinity of [^{3}H]methylscopolamine([^{3}H]MS) binding, but did not affect the number of [^{3}H]MS binding sites in the rat brainstem. Isoflurane inhibits muscarinic receptor-evoked cyclic GMP production in cultured bovine adrenal medullary cells, suggesting that isoflurane inhibits M1R [18]. Lin et al. [19] showed that enflurane inhibits the function of mouse and human brain phosphatidylinositol-linked MRs expressed in *Xenopus* oocytes.

There have been several reports on the effects of volatile anesthetics on recombinant MRs using the *Xenopus* oocyte expression system. Halothane inhibits signaling via M1R expressed in *Xenopus* oocytes [6–12]. Desflurane has a biphasic effect on M1R signaling, enhancing it at lower concentrations, but depressing it at higher concentrations and a similar, although not significant, trend was observed with M3R signaling [20]. Isoflurane has no effect on M1R signaling, but inhibits M3R signaling [20, 21]. Sevoflurane depresses the function of M1R and M3R signaling in a dose-dependent manner [22]. Similar to its known effect on M1R signaling, halothane also depresses M3R function dose-dependently [22].

There are several reports on the inhibitory effects of intravenous anesthetics, ketamine, propofol, thiopental, alphaxalone and an α_2-adrenoceptor agonist, dexmedetomidine. Durieux [23] reported that ketamine profoundly inhibits muscarinic signaling. Nagase et al. [24] reported that propofol inhibits M1R-mediated signal transduction at the receptor site or the site of interaction between the receptor and associated G proteins. Shiraishi et al. [25] recently reported the inhibitory effects of alphaxalone on M1R and M3R expressed in *Xenopus* oocytes. Dexmedetomidine has little effect on the M1R function expressing in

Table 3 The effects of Intravenous anesthetics and analgesics on Gq coupled receptor function

	Dex.	Ketamine	Propofol	Pent.	Alph.	Tramadol	ODT
M_1	→	↓	↓		↓	↓	↓
M_3	↓	↓			↓	↓	→
$5HT_{2A}$		→	→	→			
$5HT_{2C}$	→					↓	↓
mGluR1							
mGluR5							
Substance P	→	↓	→	↓		→	↓
Orexin 1	→	↓		↓			

Dex. dexmedetomidine, *Pent.* pentobarbiturate, *Alph.* alfaxalone, *ODT O*-desmethyl tramadol

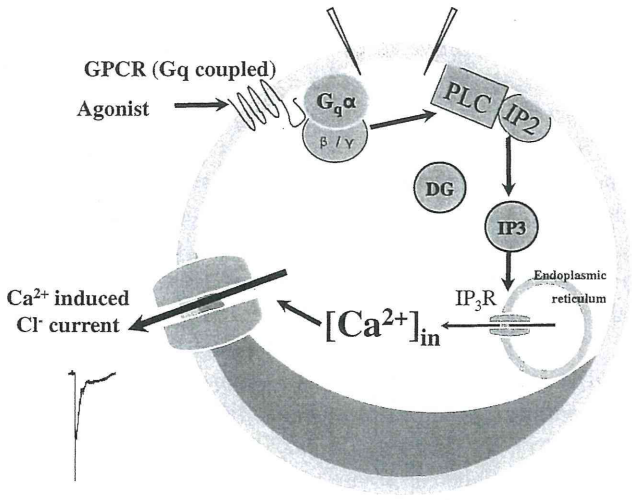


Fig. 2 Intracellular signaling of Gq coupled receptor expressing in *Xenopus* oocytes. Stimulation of G_q coupled receptors results in activation of Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes; stimulation of G_q coupled receptors leads to G protein-dependent activation of phospholipase C, resulting in the formation of IP_3 and diacylglycerol. The IP_3 causes the release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes

Xenopus oocytes expressing M1Rs. In contrast, dexmedetomidine inhibited the ACh-induced currents in *Xenopus* oocytes expressing M3Rs [26].

Local anesthetics also inhibit MRs. Clinically relevant concentrations of lidocaine inhibit M1R signaling [27–29]. Hollmann et al. [27–30] suggested that the major site of action is an extracellular domain of the muscarinic receptor; the N-terminus and third extracellular loop of the M1R molecule were identified as necessary for extracellular inhibition by charged LA, and the intracellular effect of LA most likely takes place at the $G_{\alpha q}$-subunit.

There have been several reports with evidence that showed the effects of analgesics. The effects of tramadol on MRs have been well studied. Information on the effects of tramadol on MRs is scarce. In a rat brain binding experiment, Frink et al. [31] showed that tramadol and its metabolite, *O*-desmethyl tramadol (ODT), have no affinity for M1R. We investigated the effects of tramadol on M1R in two different systems, a *Xenopus* oocyte expression system and on cultured bovine adrenal medullary cells. Tramadol competitively inhibited acetylcholine (ACh)-induced currents in *Xenopus* oocytes expressing the M1R [32]. In cultured bovine adrenal medullary cells, tramadol suppressed muscarine-induced cyclic GMP accumulation and inhibited the specific binding of [^{3}H]-quinuclidinyl benzilate (QNB) [32]. These findings suggest that tramadol inhibits MR function via QNB-binding sites. We also investigated the effects of tramadol on M3R using the *Xenopus* oocytes expression system [33]. Tramadol inhibited ACh-induced currents in oocytes expressing the M3R and the specific binding of [^{3}H]-QNB, suggesting that tramadol inhibits M3R function via QNB-binding sites. This may explain the modulation of neuronal function and the anticholinergic effects of tramadol in clinical situations. To confirm the anticholinergic action of tramadol, we investigated the effects of tramadol on the pH of gastric juices during anesthesia in order to determine whether tramadol inhibits the secretion of gastric juices from gastric glands [34]. After anesthesia was induced, the gastric pH was measured using pH test paper; then tramadol (100 mg), famotidine (20 mg), or saline was injected into the deltoid muscle. The gastric pH was increased by the same amount in both the tramadol and famotidine groups at 3 h after drug administration, suggesting that tramadol inhibits the secretion of gastric acid. The effects of the metabolite ODT on M1R and M3R functions in the *Xenopus* oocytes expression system have been reported [35]; the inhibitory effects of ODT on muscarinic receptors are different from those of tramadol. ODT inhibits M1R function but has little effect on M3R function [35].

As mentioned above, there is much evidence that MRs are the targets of anesthetics and analgesics. By contrast, a recent report pointed out that MRs do not seem to mediate the immobilization caused by inhaled anesthetics [36]. Previous studies have focused on G_q-coupled receptors (M1R and M3R), although, there has been little information on the other MRs, such as M2R. More studies are necessary to reveal the roles of individual MRs in the mechanisms of anesthetics and analgesics.

5-Hydroxytryptamine (5-HT; serotonin) receptors

5-Hydroxytryptamine (5-HT; serotonin) is a neurotransmitter that is essential for a large number of physiological processes, including the regulation of vascular and non-vascular smooth muscle contraction, modulation of platelet aggregation, and the regulation of appetite, mood, anxiety, wakefulness, and perception [37, 38]. To mediate this astonishing array of functions, no fewer than 15 separate receptors have evolved, of which all but two (5-HT_{3A} and 5-HT_{3B}) are GPCRs [37, 38].

Although seven different families of 5-HT receptors (5-HTR) have been identified, there is little information on the effects of anesthetics on G-protein-coupled 5-HTRs. Several investigators recently studied the effects of anesthetics on two types of metabotropic 5HTR. Enflurane inhibited the function of phosphatidylinositol-linked acetylcholine and 5-HTR [19]. We previously reported the inhibitory effects of anesthetics on 5-HT_{2A}R in detail. Halothane decreased 5-HT_{2A}R-mediated responses in a concentration-dependent manner, and the inhibitory effects of halothane were attenuated by treatment with the protein kinase C (PKC) inhibitor GF109203X. These findings imply that metabotropic 5-HTRs are affected by halothane, and that these actions may be dependent on the activity of PKC.

By contrast, the intravenous anesthetics propofol, ketamine, pentobarbital, and etomidate did not affect the functions of 5-HT_{2A}R. Dexmedetomidine has little effect on the 5-HT_{2C} receptors function expressing in *Xenopus* oocytes [7]. Tramadol inhibited 5-HT-induced Cl^- currents at pharmacologically relevant concentrations, and the mechanism of this inhibitory effect seems to involve competitive displacement of the 5-HT binding to the 5-HT_{2C}R, rather than via activation of the PKC pathway. ODT is a more potent analgesic than tramadol. ODT, at pharmacologically relevant concentrations, inhibited 5-HT-evoked Ca^{2+}-activated Cl^- currents in oocytes that expressed 5-HT_{2C}R. ODT inhibited the specific binding of [^{3}H]5-HT by 5-HT_{2C}R expressed in oocytes. ODT altered the apparent dissociation constant for binding of [^{3}H]5-HT by 5-HT_{2C}R without changing maximum binding, which indicated competitive inhibition [39].

There have been several findings with evidence that 5-HTR is a one of the targets of volatile anesthetics, but intravenous anesthetics do not seem to have an effect on them. By contrast, a recent report pointed out that tramadol and metabolite ODT would have inhibition.

Substance P receptors

Substance P receptors (SPR) are widely distributed in the CNS and peripheral nerves. SP is a neurotransmitter that is released from C-fibers within nociceptive primary afferent neurons to the spinal cord and mediates part of the excitatory synaptic input to nociceptive neurons at this level [40–42]. A recent study of mice lacking the gene encoding SPR showed that the mice had altered pain sensitivity; nociceptive responses to certain somatic and visceral noxious stimuli are reduced in SPR knockout mice [43–45]. Accordingly, much attention has been paid to the role of SPR in anesthetic mechanisms.

Recently, we reported the effects of halothane, isoflurane, enflurane, and diethyl ether on substance P-induced currents mediated by SPR expressed in *Xenopus* oocytes [9]. All of the volatile anesthetics tested inhibited SPR-induced Ca^{2+}-activated Cl^- currents at pharmacologically relevant concentrations. The PKC inhibitor GF109203X enhanced the substance P-induced Cl^- currents. However, GF109203X abolished the inhibitory effects of the volatile anesthetics examined on SPR. These results demonstrate that halothane, isoflurane, enflurane and diethyl ether inhibit the function of SPR and suggest that activation of PKC is involved in the mechanism of action of anesthetics and ethanol on the inhibitory effects of SPR.

The intravenous anesthetics ketamine and pentobarbital inhibit SPR-induced currents at pharmacologically relevant concentrations, while propofol has little effect on the currents [46]. By contrast, GF109203X did not abolish the inhibitory effects of ketamine and pentobarbital on substance P-induced Ca^{2+}-activated Cl^- currents. Moreover, ketamine and pentobarbital inhibited the specific binding of [^{3}H]-substance P to SPR expressed in *Xenopus* oocytes. Scatchard analysis of [^{3}H]SP binding revealed that ketamine and pentobarbital decreased the apparent dissociation constant for binding and maximal binding, indicating noncompetitive inhibition. The results suggest that ketamine and pentobarbital inhibit SPR function.

In contrast to anesthetics, there has been little information about the effects of analgesics on SPR. Tramadol has little effect on SPR expressed in *Xenopus* oocytes [46]. On the other hand, we recently reported that inhibitory effects of ODT have much greater analgesic potency than tramadol itself on SPR [47]. In this study, we investigated the effects of ODT on SPR expressed in *Xenopus* oocytes by examining substance P-induced Ca^{2+}-activated Cl^- currents. ODT inhibited the SPR-induced Cl^- currents at pharmacologically relevant concentrations, however, GF109203X did not abolish the inhibitory effects of ODT on SP-induced Ca^{2+}-activated Cl^- currents. The results suggest that the ODT inhibits the SPR functions, which may be independent of activation of PKC-mediated pathways.

These findings imply that SPR are affected by most volatile anesthetics and some intravenous anesthetics. Propofol and tramadol have little effect on the currents.

However, ketamine, pentobarbital and ODT have inhibitory effects. The mechanisms of inhibition by intravenous anesthetics are different from those of volatile anesthetics.

Metabotropic glutamate receptors

The metabotropic glutamate receptors (mGluRs) are distinct from the other metabotropic receptors in that they are much larger proteins and show little sequence similarity to most members of the GPCR family, although there is appreciable homology with the γ-aminobutyric acid B ($GABA_B$) receptors [48]. The mGluRs are important modulators of synaptic transmission in the mammalian CNS, and are believed to play roles in memory and learning. Therefore, it was of interest to determine whether anesthetics and analgesics affect the function of these receptors. The mGluRs form a family of receptors with eight different subtypes (mGluR1-8 [49, 50]). Based on their pharmacology, second messenger coupling, and sequence differences, these receptors can be divided into Classes I (mGluR1 and mGluR5), II (mGluR2 and mGluR3), and III (mGluR4 and mGluR6-8) [51]. The Class I receptors are linked to learning and memory [51] in pharmacological studies showing that an agonist of mGluR1 and mGluR5 enhances memory and that mutant mice lacking mGluR1 show deficits in learning and memory and reduced hippocampal LTP [51–53]. Mice lacking mGluR1 also display poor motor coordination. More recently, it was reported that mice lacking mGluR5 show impaired learning and reduced CA1 LTP, but normal CA3 LTP [54]. In view of the effects of ethanol and anesthetics on learning, memory, and motor function, it is of interest to consider the effects of these drugs on Class I mGluRs.

There are few studies of the effects of anesthetic agents on mGluRs. Ethanol inhibits quisqualate-induced burst activity in rat cultured cerebellar Purkinje neurons, which are mediated by mGluRs [55], and quisqualate-induced currents in *Xenopus* oocytes expressing mRNA from rat cerebellum [56], but does not affect the glutamate-stimulated phospholipase C activity of brain astrocytes [57]. These results suggest that ethanol inhibits some, but not all, mGluR subtypes, and encouraged us to test the effects of ethanol and anesthetics on specific mGluR subtypes expressed in *Xenopus* oocytes.

The effects of ethanol and halothane, on the function of mGluR1 and mGluR5 expressed in *Xenopus* oocytes have been reported [8]. Halothane and ethanol inhibited mGluR5-induced Ca^{2+}-activated Cl^- currents, yet pharmacologically relevant concentrations of these compounds had little effect on the glutamate-induced currents in the oocytes expressing mGluR1. The GF109203X abolished the inhibitory effects of halothane and ethanol on mGluR5s. Conversely, the phosphatase inhibitor calyculin A prolonged the actions of halothane and ethanol. Furthermore, mutation of a PKC consensus site (Ser890) of mGluR5 abolished the inhibitory effects of halothane and ethanol. These results suggest that ethanol and volatile anesthetics inhibit mGluR5 because they promote PKC-mediated phosphorylation.

Orexin A receptor

Neurons in the hypothalamus containing the neuropeptide orexin have been implicated in the control of sleep and wakefulness and in the pathology of narcolepsy. We have investigated the effects of volatile anesthetics, ethanol and intravenous anesthetics on orexin-A-induced Ca^{2+}-activated Cl^- currents using *Xenopus* oocytes expressing orexin-1 receptors (OX1Rs) [58]. The volatile anesthetics isoflurane, enflurane and halothane inhibited Cl^- currents elicited by 1-micromol/l orexin-A. Pentobarbital and ketamine also inhibited the action of orexin-A. Dexmedetomidine had little inhibition on the orexin A-induced current in oocytes expressing the OX1Rs [26]. Although more study would be necessary, these results may, at least in part, explain the hypnotic effects of these anesthetics.

The effect of anesthetics and analgesics on G_i protein coupled receptors

The targets of anesthetics and analgesics are not only G_q coupled receptors; other GPCRs can be targets as well. There has been little information regarding G_s- and G_i-coupled receptors, especially. Especially, the G_i coupled receptors, have been thought of as one of the sites of the anesthetics and analgesics. However, historically there have not been convenient assay systems to study the effects of anesthetics and analgesics on G_i coupled receptors. Because stimulation of G_i coupled receptors results in inhibition of cyclic AMP in cells and dose does not affect the Ca^{2+} elevation; the system has been well characterized, and has proven useful for studying the effects of intravenous anesthetics on GPCRs.

Recently, several investigators reported assay systems using chimera G proteins between G_i and G_q for investigation of G_i coupled receptors (Table 4). Coward et al. [59], have reported that the chimeric G proteins alter

Table 4 The effects of anesthetics on Gi coupled receptor function

	Halothane	Sevoflurane	Ketamine	Propofol
M_2	↓			
μ opioid	↓	↓	↓	→

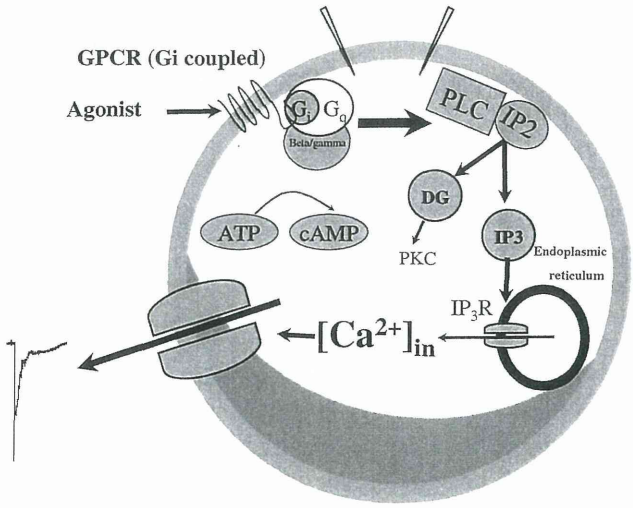


Fig. 3 Intracellular signaling in *Xenopus* oocytes expressing a Gi coupled receptor fused to chimeric Gα protein Gq_{i5}. Stimulation of $G_{i/o}$ coupled receptor fused to Gq_{i5} leads to Gq_{i5} protein-dependent activation of phospholipase C, resulting in the formation of IP_3 and diacylglycerol. The IP_3 causes the release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes

receptor coupling so that signaling can occur through G_q and result in mobilization of intracellular calcium stores in CHO cells. From this evidence, we drew a hypothesis that it may be able to used for the analysis by injecting both cRNA the $G_{i/q}$ chimaeric G proteins ($G_{i/q}$) and M2R into the *Xenopus* oocytes as well as G_q coupled receptors. To establish the system, we investigated whether we could measure if the acetylcholine stimulation leads to activation of phospholipase C, resulting in the formation of IP_3 and diacylglycerol and the release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes which were injected both cRNA and the $G_{i/q}$ chimera G proteins and G_i coupled receptor, M2R [60] (Table 4). However, regarding the established analysis of injecting both cRNA the $G_{i/q}$ chimaeric G proteins and M2R into the *Xenopus* oocytes as well as G_q coupled receptors, there were some problems for assay, i.e, low expression rates and low evoking currents. Recently, in order to improve the $G_{i/o}$-coupled receptor assay system, we made a $G_{i/o}$-coupled receptor fused to Gq_{i5} (μOR-G_{qi5}) and expressed it in *Xenopus* oocytes [61, 62] (Fig. 3). By using this assay system, we examined the effects of anesthetics on the function of μOR (Table 4).

The effects of anesthetics and on M2R

We examined ACh-induced Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes coexpressing G_i-coupled M2R with the chimeric $G\alpha_{qi5}$ [60]. In oocytes coexpressing M2R and $G\alpha_{qi5}$, halothane inhibited M2R -induced Cl^- currents in a concentration-dependent manner, suggesting that halothane inhibits M_2R-induced cellular responses at clinically relevant concentrations. Treatment with the PKC inhibitor GF109203X produced a 3.5-fold enhancement of the initial Cl^- currents induced by ACh in oocytes expressing M2R and G_{qi5}. The rate of halothane-induced inhibition of Cl^- currents elicited by ACh, however, was not changed in such oocytes pretreated with GF109203X. These findings suggest that halothane inhibits the M2R-induced signaling by acting at sites other than PKC activity.

The effects of anesthetics and analgesics on opioid receptors

Opioids are commonly used analgesics in clinical practice, however, the actual role of opioid receptors (OR) in anesthetic action have been unclear. It has been reported that OR antagonist naloxone does not affect the anesthetic potency of halothane in animals [63, 64]. Moreover, naloxone dose does not antagonize the analgesic effects of inhalation anesthetics [65]. On the other hand, Sarton et al. [66], reported that S(+) ketamine interacts with the μ-opioid system at supraspinal sites. In order to make clear the role of ORs in anesthetic action, it would be necessary to study the direct effects on OR functions.

The ORs belong to the GPCR family and three types of opioid receptors, μ, δ and κ, have been identified by molecular cloning [67]. Within three subtypes of these receptors, μORs are the major receptor to mediate the analgesic effects of opioids [67]. On the basis of second messenger signaling, μOR couple to the $G\alpha_{i/o}$ protein to cause inhibition of adenylate cyclase, inhibition of voltage-dependent Ca^{2+} channels or activation of G protein-coupled inwardly rectifying K^+ channels (GIRKs) [67]. Functions of G_q coupled receptors have been reported to be modified by some anesthetics and analgesics [5, 68]; as far as the functions of $G_{i/o}$-coupled receptors including μOR are concerned, much less is known about the direct effects of anesthetics and analgesics.

We and others have previously used oocytes expressing GIRK channels for the analysis of the function of $G_{i/o}$ protein-coupled receptors such as μOR, $5HT_{1A}R$, $GABA_BR$ or cannabinoid CB_1 receptors [62, 69–71]; GIRKs has been demonstrated as being reporter channels for assay of the activity of $G_{i/o}$-coupled receptors [69]. However, recent reports have revealed that GIRKs are possible targets for several anesthetics including halothane [72, 73]. Also, GIRKs have been reported to be possible targets for alcohol [74]. In such situations, it should be taken into consideration that functions of either $G_{i/o}$-coupled receptors or GIRKs, or both, could be affected by

anesthetics if GIRKs are used as reporters [72–74]. Thus, we employed a μOR fused to G_{qi5} (μOR-G_{qi5}) in the *Xenopus* oocyte expression assay system. Accordingly, this system makes possible the study of the direct effects of anesthetics on μOR functions.

In our recent report, halothane, ketamine, propofol and ethanol themselves had no effects on μOR-G_{qi5} in oocytes expressing μOR-G_{qi5} [61]. In contrast, ketamine and ethanol inhibited the [D-Ala2,N-Me-Phe4,Gly5-ol] enkephalin (DAMGO)-induced Cl^- currents at clinically equivalent concentrations. Halothane and propofol only inhibited the DAMGO-induced Cl^- currents at higher but clinically used concentrations. These findings suggest that ketamine and ethanol inhibit the μOR function directly in clinical practice.

Sevoflurane is commonly used together with opioids in clinical practice. However, the effects of sevoflurane on μOR functions are still unclear. Our recent study reveals that the effects of sevoflurane on the μOR functions were analyzed by using *Xenopus* oocytes expressing a μOR fused to chimeric Gα protein Gq_{i5} (μOR-Gq_{i5}) [75]. Sevoflurane inhibited the DAMGO-induced Cl^- currents at clinically used concentrations by PKC activation. These findings suggest that sevoflurane would inhibit the μOR function in clinical practice. The same as with sevoflurane, propofol has been commonly used together with opioids clinically. From the viewpoint of the effects of the opioid receptors function, propofol may be a better choice than sevoflurane.

Future directions

Several lines of study have shown that GPCRs are also targets for them. It has been reported that some anesthetics inhibit the functions of G_q-coupled receptors, such as M1R. Although GPCRs are the most numerous therapeutic targets known, the ligands for approximately two-thirds of these receptors remain unknown. The challenge in the post-genomic era is to evaluate the role of these orphan GPCRs (oGPCRs) in normal physiology and disease, and to develop new therapies based on this information. Many oGPCRs are expressed in the brain, suggesting the existence of unidentified neurotransmitters. Nearly 160 GPCRs have been identified based on their gene sequence and ability to interact with known endogenous ligands. However, an estimated 500–800 additional GPCRs have been classified as "orphan" receptors (oGPCRs) because their endogenous ligands have not yet been identified. Given that known GPCRs are targets for anesthetics and analgesics, these oGPCRs represent a rich group of receptor targets for them.

Previous reports show that anesthetics affect the function of GPCRs, and this suggests that some oGPCRs are the targets of anesthetics. Several oGPCRs involved in pain and nociception have been reported [76]. Majane and Yang [76] reported that neuropeptide FF (NPFF) modulated pain sensation and morphine analgesia under normal and pathological conditions, via both spinal and brain mechanisms [77]. It would be interesting to study the effects of anesthetics on oGPCRs, which modulate pain like NPFF. More information about orphan GPCRs might help to elucidate the role of GPCRs in the mechanisms of anesthetics and analgesics.

The G_s- and G_i-coupled receptors might also be targets for anesthetics. Compared with Gq coupled GPCRs, there has been less information which shows the effects of anesthetics on G_s- and G_i-coupled receptors. The new assay systems using chimeric G protein [61] could be helpful to study them and make a role to help clarify their mechanisms .

Conclusion

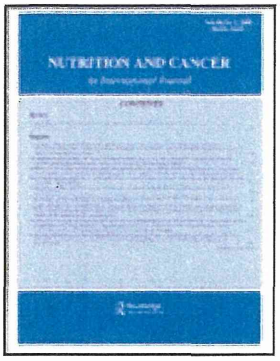
Until today, ligand-gated ion channels, such as GABA, have been believed to be the site of action of the anesthetic. The mechanisms of anesthetics on GPCRs has become more evident in recent years. The action of anesthetics on GPCRs could also play an important role for anesthetic mechanisms. In particular, the effect of anesthetics on Gq protein coupled receptors has become apparent. The effects of anesthetics on Gs and Gi protein-coupled receptors are expected to be studied more in the future. In addition, the effect of anesthetics on the orphan receptor is not clear in how it works. It would be interesting to make clear the effects of anesthetics on these receptors. More time and more research on the effects of anesthetics for GPCRs, could make clear the mechanism of action of anesthetic agents in vivo.

References

1. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature. 1994;367:607–14.
2. Harris RA, Mihic SJ, Dildy-Mayfield JE, Machu TK. Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition. FASEB J. 1995;9:1454–62.
3. Campagna JA, Miller KW, Forman SA. Mechanisms of actions of inhaled anesthetics. N Engl J Med. 2003;348:2110–24.
4. Bonin RP, Orser BA. GABA(A) receptor subtypes underlying general anesthesia. Pharmacol Biochem Behav. 2008;90:105–12.
5. Minami K, Uezono Y. G_q protein-coupled receptors as targets for anesthetics. Curr Pharm Des. 2006;12:1931–7.
6. Minami K, Vanderah TW, Minami M, Harris RA. Inhibitory effects of anesthetics and ethanol on muscarinic receptors expressed in *Xenopus* oocytes. Eur J Pharmacol. 1997;339:237–44.

7. Minami K, Minami M, Harris RA. Inhibition of 5-hydroxytryptamine type 2A receptor-induced currents by n-alcohols and anesthetics. J Pharmacol Exp Ther. 1997;281:1136–43.
8. Minami K, Gereau RW IV, Minami M, Heinemann SF, Harris RA. Effects of ethanol and anesthetics on type 1 and 5 metabotropic glutamate receptors expressed in *Xenopus* laevis oocytes. Mol Pharmacol. 1998;53:148–56.
9. Minami K, Shiraishi M, Uezono Y, Ueno S, Shigematsu A. The inhibitory effects of anesthetics and ethanol on substance P receptors expressed in *Xenopus* oocytes. Anesth Analg. 2002;94:79–83.
10. Caulfield MP. Muscarinic receptors—characterization, coupling and function. Pharmacol Ther. 1993;58:319–79.
11. Fibiger HC. Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. Trends Neurosci. 1991;14:220–3.
12. Durieux ME. Halothane inhibits signaling through m1 muscarinic receptors expressed in *Xenopus* oocytes. Anesthesiology. 1995;82:174–82.
13. Eglen RM, Choppin A, Dillon MP, Hegde S. Muscarinic receptor ligands and their therapeutic potential. Curr Opin Chem Biol. 1999;3:426–32.
14. Wess J, Blin N, Yun J, Schoneberg T, Liu J. Molecular aspects of muscarinic receptor assembly and function. Prog Brain Res. 1996;109:153–62.
15. Wess J. Molecular biology of muscarinic acetylcholine receptors. Crit Rev Neurobiol. 1996;10:69–99.
16. Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci. 1991;11:3218–26.
17. Anthony BL, Dennison RL, Aronstam RS. Disruption of muscarinic receptor-G protein coupling is a general property of liquid volatile anesthetics. Neurosci Lett. 1989;99:191–6.
18. Minami K, Yanagihara N, Toyohira Y, Tsutsui M, Shigematsu A, Wada A, Izumi F. Isoflurane inhibits nicotinic acetylcholine receptor-mediated $^{22}Na^{+}$ influx and muscarinic receptor-evoked cyclic GMP production in cultured bovine adrenal medullary cells. Naunyn Schmiedebergs Arch Pharmacol. 1994;349:223–9.
19. Lin LH, Leonard S, Harris RA. Enflurane inhibits the function of mouse and human brain phosphatidylinositol-linked acetylcholine and serotonin receptors expressed in *Xenopus* oocytes. Mol Pharmacol. 1993;43:941–8.
20. Nietgen GW, Honemann CW, Chan CK, Kamatchi GL, Durieux ME. Volatile anaesthetics have differential effects on recombinant m1 and m3 muscarinic acetylcholine receptor function. Br J Anaesth. 1998;81:569–77.
21. Do SH, Kamatchi GL, Durieux ME. The effects of isoflurane on native and chimeric muscarinic acetylcholine receptors: the role of protein kinase C. Anesth Analg. 2001;93:375–81.
22. Nietgen GW, Honemann CW, Durieux ME. Influence of anesthetics on endogenous and recombinantly expressed G protein-coupled receptors in the *Xenopus* oocyte. Toxicol Lett. 1998;100–101:319–27.
23. Durieux ME. Inhibition by ketamine of muscarinic acetylcholine receptor function. Anesth Analg. 1995;81:57–62.
24. Nagase Y, Kaibara M, Uezono Y, Izumi F, Sumikawa K, Taniyama K. Propofol inhibits muscarinic acetylcholine receptor-mediated signal transduction in *Xenopus* oocytes expressing the rat M1 receptor. Jpn J Pharmacol. 1999;79:319–25.
25. Shiraishi M, Minami K, Shibuya I, Uezono Y, Ogata J, Okamoto T, Murasaki O, Kaibara M, Ueta Y, Shigematsu A. The inhibitory effects of alphaxalone on M1 and M3 muscarinic receptors expressed in *Xenopus* oocytes. Anesth Analg. 2003;97:449–55.
26. Takizuka A, Minami K, Uezono Y, Horishita T, Yokoyama T, Shiraishi M, Sakurai T, Shigematsu A, Ueta Y. Dexmedetomidine inhibits muscarinic type 3 receptors expressed in *Xenopus* oocytes and muscarine-induced intracellular Ca^{2+} elevation in cultured rat dorsal root ganglia cells. Naunyn Schmiedebergs Arch Pharmacol. 2007;375:293–301.
27. Hollmann MW, Fischer LG, Byford AM, Durieux ME. Local anesthetic inhibition of m1 muscarinic acetylcholine signaling. Anesthesiology. 2000;93:497–509.
28. Hollmann MW, Wieczorek KS, Berger A, Durieux ME. Local anesthetic inhibition of G protein-coupled receptor signaling by interference with Gα(q) protein function. Mol Pharmacol. 2001;59:294–301.
29. Hollmann MW, Ritter CH, Henle P, de Klaver M, Kamatchi GL, Durieux ME. Inhibition of m3 muscarinic acetylcholine receptors by local anaesthetics. Br J Pharmacol. 2001;133:207–16.
30. Hollmann MW, Difazio CA, Durieux ME. Ca-signaling G-protein-coupled receptors: a new site of local anesthetic action? Reg Anesth Pain Med. 2001;26:565–71.
31. Frink MC, Hennies HH, Englberger W, Haurand M, Wilffert B. Influence of tramadol on neurotransmitter systems of the rat brain. Arzneimittelforschung. 1996;46:1029–36.
32. Shiraishi M, Minami K, Uezono Y, Yanagihara N, Shigematsu A. Inhibition by tramadol of muscarinic receptor-induced responses in cultured adrenal medullary cells and in *Xenopus* laevis oocytes expressing cloned M1 receptors. J Pharmacol Exp Ther. 2001;299:255–60.
33. Shiga Y, Minami K, Shiraishi M, Uezono Y, Murasaki O, Kaibara M, Shigematsu A. The inhibitory effects of tramadol on muscarinic receptor-induced responses in *Xenopus* oocytes expressing cloned M(3) receptors. Anesth Analg. 2002;95:1269–73.
34. Minami K, Ogata J, Horishita T, Shiraishi M, Okamoto T, Sata T, Shigematsu A. Intramuscular tramadol increases gastric pH during anesthesia. Can J Anaesth. 2004;51:545–8.
35. Nakamura M, Minami K, Uezono Y, Horishita T, Ogata J, Shiraishi M, Okamoto T, Terada T, Sata T. The effects of the tramadol metabolite *O*-desmethyl tramadol on muscarinic receptor-induced responses in *Xenopus* oocytes expressing cloned M1 or M3 receptors. Anesth Analg. 2005;101:180–6.
36. Eger EI 2nd, Zhang Y, Laster M, Flood P, Kendig JJ, Sonner JM. Acetylcholine receptors do not mediate the immobilization produced by inhaled anesthetics. Anesth Analg. 2002;94:1500–4.
37. Gyermek L. Pharmacology of serotonin as related to anesthesia. J Clin Anesth. 1996;8:402–25.
38. Kroeze WK, Kristiansen K, Roth BL. Molecular biology of serotonin receptors structure and function at the molecular level. Curr Top Med Chem. 2002;2:507–28.
39. Horishita T, Minami K, Uezono Y, Shiraishi M, Ogata J, Okamoto T, Shigematsu A. The tramadol metabolite, *O*-desmethyl tramadol, inhibits 5-hydroxytryptamine type 2C receptors expressed in *Xenopus* oocytes. Pharmacology. 2006;77:93–9.
40. De Koninck Y, Henry JL. Substance P-mediated slow excitatory postsynaptic potential elicited in dorsal horn neurons in vivo by noxious stimulation. Proc Natl Acad Sci USA. 1991;88:11344–8.
41. Xu XJ, Wiesenfeld-Hallin Z. Intrathecal neurokinin A facilitates the spinal nociceptive flexor reflex evoked by thermal and mechanical stimuli and synergistically interacts with substance P. Acta Physiol Scand. 1992;144:163–8.
42. Chapman V, Dickenson AH. The effect of intrathecal administration of RP67580, a potent neurokinin 1 antagonist on nociceptive transmission in the rat spinal cord. Neurosci Lett. 1993;157:149–52.
43. Cao YQ, Mantyh PW, Carlson EJ, Gillespie AM, Epstein CJ, Basbaum AI. Primary afferent tachykinins are required to experience moderate to intense pain. Nature. 1998;392:390–4.
44. Zimmer A, Zimmer AM, Baffi J, Usdin T, Reynolds K, König M, Palkovits M, Merey E. Hypoalgesia in mice with a targeted deletion of the tachykinin 1 gene. Proc Natl Acad Sci USA. 1998;95:2630–5.

45. De Felipe C, Herrero JF, O'Brien JA, Palmer JA, Doyle CA, Smith AJ, Laird JM, Belmonte C, Cervero F, Hunt SP. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature. 1998;392:394–7.
46. Okamoto T, Minami K, Uezono Y, Ogata J, Shiraishi M, Shigematsu A, Ueta Y. The inhibitory effects of ketamine and pentobarbital on substance p receptors expressed in *Xenopus* oocytes. Anesth Analg. 2003;97:104–10.
47. Minami K, Yokoyama T, Ogata J, Uezono Y. The tramadol metabolite *O*-desmethyl tramadol inhibits substance P-receptor functions expressed in *Xenopus* oocytes. J Pharmacol Sci. 2011; 115:421–4.
48. Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, Bettler B. Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. Nature. 1997;386:239–46.
49. Abe T, Sugihara H, Nawa H, Shigemoto R, Mizuno N, Nakanishi S. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca^{2+} signal transduction. J Biol Chem. 1992;267:13361–8.
50. Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol. 1997;37: 205–37.
51. Riedel G. Function of metabotropic glutamate receptors in learning and memory. Trends Neurosci. 1996;19:219–24.
52. Aiba A, Chen C, Herrup K, Rosenmund C, Stevens CF, Tonegawa S. Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. Cell. 1994;79:365–75.
53. Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, Bordi F, Franz-Bacon K, Reggiani A, Matarese V, Condé F, Collingridge GL, Crépel F. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. Nature. 1994;372:237–43.
54. Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci. 1997;17:5196–205.
55. Netzeband JG, Gruol DL. Modulatory effects of acute ethanol on metabotropic glutamate responses in cultured Purkinje neurons. Brain Res. 1995;688:105–13.
56. Sanna E, Dildy-Mayfield JE, Harris RA. Ethanol inhibits the function of 5-hydroxytryptamine type 1c and muscarinic M1 G protein-linked receptors in *Xenopus* oocytes expressing brain mRNA: role of protein kinase C. Mol Pharmacol. 1994;45: 1004–12.
57. Smith TL. Selective effects of ethanol exposure on metabotropic glutamate receptor and guanine nucleotide stimulated phospholipase C activity in primary cultures of astrocytes. Alcohol. 1994;11:405–9.
58. Minami K, Uezono Y, Sakurai T, Horishita T, Shiraishi M, Ueta Y. Effects of anesthetics on the function of orexin-1 receptors expressed in *Xenopus* oocytes. Pharmacology. 2007;79:236–42.
59. Coward P, Chan SD, Wada HG, Humphries GM, Conklin BR. Chimeric G proteins allow a high-throughput signaling assay of G_i-coupled receptors. Anal Biochem. 1999;270:242–8.
60. Minami K, Uezono Y, Shiraishi M, Okamoto T, Ogata J, Horishita T, Taniyama K, Shigematsu A. Analysis of the effects of halothane on G_i-coupled muscarinic M2 receptor signaling in *Xenopus* oocytes using a chimeric G alpha protein. Pharmacology. 2004;72:205–12.
61. Minami K, Sudo Y, Shiraishi S, Seo M, Uezono Y. Analysis of the effects of anesthetics and ethanol on μ-opioid receptor. J Pharmacol Sci. 2010;112:424–31.
62. Hojo M, Sudo Y, Ando Y, Minami K, Takada M, Matsubara T, Kanaide M, Taniyama K, Sumikawa K, Uezono Y. μ-Opioid receptor forms a functional heterodimer with cannabinoid CB_1 receptor: electrophysiological and FRET assay analysis. J Pharmacol Sci. 2008;108:308–19.
63. Harper MH, Winter PM, Johnson BH, Eger EI 2nd. Naloxone does not antagonize general anesthesia in the rat. Anesthesiology. 1978;49:3–5.
64. Pace NL, Wong KC. Failure of naloxone and naltrexone to antagonize halothane anesthesia in the dog. Anesth Analg. 1979;58:36–9.
65. Levine LL, Winter PM, Nemoto EM, Uram M, Lin MR. Naloxone does not antagonize the analgesic effects of inhalation anesthetics. Anesth Analg. 1986;65:330–2.
66. Sarton E, Teppema LJ, Olievier C, Nieuwenhuijs D, Matthes HW, Kieffer BL, Dahan A. The involvement of the mu-opioid receptor in ketamine-induced respiratory depression and antinociception. Anesth Analg. 2001;93:1495–500.
67. Surratt CK, Adams WR. G protein-coupled receptor structural motifs: relevance to the opioid receptors. Curr Top Med Chem. 2005;5:315–24.
68. Minami K, Uezono Y, Ueta Y. Pharmacological aspects of the effects of tramadol on G-protein coupled receptors. J Pharmacol Sci. 2007;103:253–60.
69. Dascal N. Signalling via the G protein-activated K+ channels. Cell Signal. 1997;9:551–73.
70. Uezono Y, Akihara M, Kaibara M, Kawano C, Shibuya I, Ueda Y, Yanagihara N, Toyohira Y, Yamashita H, Taniyama K, Izumi F. Activation of inwardly rectifying K^+ channels by GABA-B receptors expressed in *Xenopus* oocytes. NeuroReport. 1998;9: 583–7.
71. Ho BY, Uezono Y, Takada S, Takase I, Izumi F. Coupling of the expressed cannabinoid CB_1 and CB_2 receptors to phospholipase C and G protein-coupled inwardly rectifying K+ channels. Recept Channels. 1999;6:363–74.
72. Weigl LG, Schreibmayer W. G protein-gated inwardly rectifying potassium channels are targets for volatile anesthetics. Mol Pharmacol. 2001;60:282–9.
73. Yamakura T, Lewohl JM, Harris RA. Differential effects of general anesthetics on G protein-coupled inwardly rectifying and other potassium channels. Anesthesiology. 2001;95:144–53.
74. Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA. G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nat Neurosci. 1999;2:1084–90.
75. Minami K, Sudo Y, Yokoyama T, Ogata J, Takeuchi M, Uezono Y. Sevoflurane inhibits the μ-opioid receptor function expressed in *Xenopus* oocytes. Pharamacology. 2011;88:127–32.
76. Majane EA, Yang HY. Distribution and characterization of two putative endogenous opioid antagonist peptides in bovine brain. Peptides. 1987;8:657–62.
77. Panula P, Kalso E, Nieminen M, Kontinen VK, Brandt A, Pertovaara A. Neuropeptide FF and modulation of pain. Brain Res. 1999;848:191–6.



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Inhibitory Effects of Isoflavones on Tumor Growth and Cachexia in Newly Established Cachectic Mouse Models Carrying Human Stomach Cancers

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Cachexia, a negative prognostic factor, worsens a patient's quality of life. We established 2 novel cachexia models with the human stomach cancer cell line MKN-45, which was subcloned to produce potent cachexia-inducing cells by repeating the xenografts in immune-deficient mice. After subsequent xenografts, we isolated potent cachexia-inducing cells (MKN45cl85 and 85As2mLuc). Xenografts of MKN45cl85 cells in mice led to substantial weight loss and reduced adipose tissue and musculature volumes, whereas xenografts of 85As2mLuc cells resulted in highly metastatic and cachectic mice. Surgical removal of tumor tissues helped the mice regain body-weight in both mouse models. In vitro studies using these cells showed that isoflavones reduced their proliferation, implying that the isoflavones possess antiproliferative effects of these cancer cell lines. Isoflavone treatment on the models induced tumor cytostasis, attenuation of cachexia, and prolonged survival whereas discontinuation of the treatment resulted in progressive tumor growth and weight loss. The inhibitory effects of tumor growth and weight loss by isoflavones were graded as soy isoflavone aglycone AglyMax > daidzein > genistein. These results demonstrated that the 2 novel cachectic mouse models appear useful for analyzing the mechanism of cancer cachexia and monitoring the efficacy of anticachectic agents.

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INTRODUCTION

Cancer cachexia, which is characterized by loss of musculature and fat tissue, anorexia, asthenia, and anemia, makes