Molecular and cellular mechanisms of general anaesthesia

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General anaesthetics are much more selective than is usually appreciated and may act by binding to only a small number of targets in the central nervous system. At surgical concentrations their principal effects are on ligand-gated (rather than voltage-gated) ion channels, with potentiation of postsynaptic inhibitory channel activity best fitting the pharmacological profile observed in general anaesthesia. Although the role of second messengers remains uncertain, it is now clear that anaesthetics act directly on proteins rather than on lipids.

ALTHOUGH there is little agreement about how and where general anaesthetics act, there has been an explosion of studies over the past decade on the effects of anaesthetics on putative targets in the central nervous system (CNS). Partly for historical reasons, but also because of the central role membranes play in nerve conduction, attention has focused largely on nerve membranes, and in particular on neuronal ion channels and the systems that regulate them. General anaesthetics probably exert their effects on synaptic transmission rather than on axonal conduction, but which synapses are the most sensitive? Do anaesthetics principally inhibit excitatory synapses or potentiate inhibitory synapses (or both), and are the crucial targets presynaptic or postsynaptic? Moreover, do anaesthetics bind directly to proteins or influence activity by indirectly perturbing membrane lipids, and to what extent do intracellular factors modulate sensitivity? Here we try to answer these questions, concentrating on work that over the past ten years has seemed to us to be not only the most definitive but also the most pertinent in pointing the way forward.

Voltage-gated ion channels

Na^+ and K^+ channels. The interactions of many simple organic compounds with the Na^+ and K^+ channels involved in action potential generation have been described in great detail for the squid giant axon[1]. For the Na^+ channel, most anaesthetics cause depolarizing shifts in the steady-state activation curve and hyperpolarizing shifts in the steady-state inactivation curve. At concentrations relevant to general anaesthesia (Box 1), however, these shifts are very small, and, as the delayed rectifier K^+ channel is similarly insensitive[1,2], axonal conduction is virtually unaffected. Another voltage-gated channel, K_A, which is thought to play a role in regulating axonal firing, has also been proposed as a possible anaesthetic target[3]. This suggestion was made because mutants of *Drosophila* with dysfunctional K_A channels are less sensitive to isoflurane[3]. This change in anaesthetic potency, however, most probably arises from a generalized increase in neuronal excitability, because direct measurements on the K_A channel show little effect at relevant anaesthetic doses of halothane[4] or the *n*-alcohols[5]. Although it is conceivable that small perturbations of voltage-gated channels may alter patterns of neuronal firing[6,7], it appears unlikely from the available evidence that voltage-gated Na^+ or K^+ channels play a substantial role in the production of the anaesthetic state.

Ca^{2+} channels. Voltage-gated Ca^{2+} channels (currently labelled T, L, N and P) share sequence homologies with voltage-gated Na^+ and K^+ channels and belong to the same superfamily. T- and L-type channels are present in a wide variety of both excitable and non-excitable cells, but N- and P-type channels have been found mainly in neurons. To date, most anaesthetic studies have focused on T- and L-type channels, rather than on the N- and P-types. But the latter two types of Ca^{2+} channel, particularly the P-type, appear to be most directly involved in the presynaptic release of neurotransmitters from central nerve terminals[8]. The inhibitory effects of volatile anaesthetics on Ca^{2+} channels have recently been reviewed[9]; perhaps surprisingly, most studies show that these channels are either very or moderately insensitive to these agents. An apparent exception is a recent report[10] of exceptionally high sensitivity to halothane of a small low-voltage-activated Ca^{2+} current in rat sensory neurons. Barbiturates also inhibit Ca^{2+} channels[11-13], but usually with half-maximal inhibitory concentrations (IC_{50}) many-fold in excess of the free aqueous concentrations that cause general anaesthesia (Box 1). For example, the inhibitory effects of six barbiturates on N-like currents were studied[13] in *Xenopus* oocytes injected with RNA from human brain; substantial effects were found only at very high barbiturate levels (for example, for pentobarbital, $IC_{50} \approx 1$ mM and 0.4 mM for peak and sustained currents, respectively). As nerve terminal Ca^{2+}

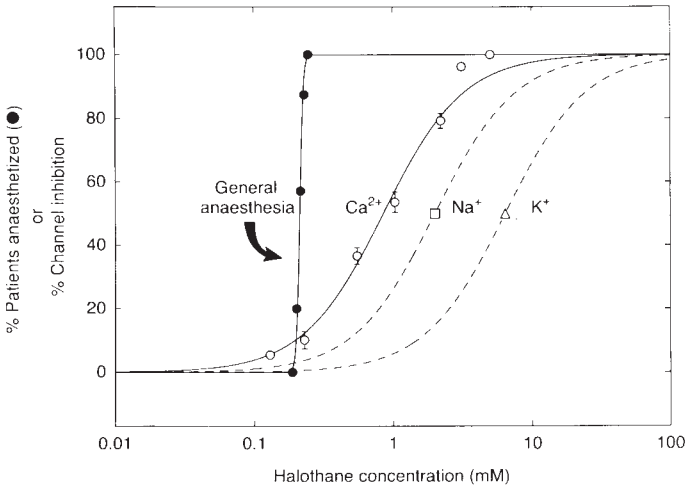


FIG. 1 Most voltage-gated ion channels are relatively insensitive to general anaesthetics. General anaesthesia in humans (●), as measured by the lack of a purposeful response to a surgical incision[128], occurs at concentrations of halothane 4 to 30 times lower than the EC_{50} concentrations needed to half-inhibit peak currents through L-type Ca^{2+} channels (○) from clonal pituitary cells[129]. Na^+ channels (□) from the squid giant axon[2] or delayed rectifier K^+ channels (△) from the squid giant axon[2]. Note the extremely steep dose–response curve for general anaesthesia. Aqueous concentrations for general anaesthesia were calculated from partial pressures[128] using known water/gas partition coefficients. Dose–response curves for the ion channels were constructed using the Hill equation with EC_{50} concentrations for the Ca^{2+}, Na^+ and K^+ channels of 0.85 mM[129], 2.0 mM[2] and 6.4 mM[2], respectively, and a Hill coefficient[129] of 1.5. The dashed lines for the Na^+ and K^+ channels reflect the fact that only the EC_{50} values are known, and for simplicity we have assumed the Hill coefficients to be the same as that for the Ca^{2+} channel.

channels open only briefly when an action potential invades a terminal, inhibition of the peak current is probably the more physiologically relevant measure of channel activity.

Overall, then, voltage-gated Ca^{2+} channels appear to be generally insensitive to clinically used general anaesthetics. It seems unlikely to us that the small inhibitions sometimes observed at surgical concentrations (Fig. 1) are sufficient to produce general anaesthesia (though the steep dependence of synaptic transmission on Ca^{2+} entry leaves this possibility open[14]). Moreover, some synapses appear to be unaffected by relevant levels of anaesthetics (see ref. 6, for example). Another approach used to assess the relevance of Ca^{2+} channels in anaesthesia relies on measurements of anaesthetic potencies in animals. For example, in both mice[15] and dogs[16], administration of Ca^{2+}-channel-blocking drugs reduces the anaesthetic requirement. Although it might be concluded that Ca^{2+} channels must therefore play a role in anaesthesia, this view must be set against the general insensitivity of these channels to anaesthetics. This illustrates the weakness of the approach, because changes in anaesthetic potencies in animals can so easily be caused by parallel effects on pathways quite distinct from those targeted by the anaesthetics themselves.

BOX 1 Which anaesthetic concentrations are pharmacologically relevant?

GENERAL anaesthetics are administered in both the gaseous and aqueous phases. A problem encountered with inhalational agents is that EC_{50}s expressed as partial pressures (usually as % atm.) are extremely temperature-dependent, with the gas-phase potencies almost invariably increasing as the temperature is lowered. In fact, an anaesthetic partial pressure determined for a mammal at body temperature can be as much as three times too high for an experimental preparation at 20 °C (ref. 9). But as there is usually a parallel (although slightly smaller) temperature-dependence of water/gas partitioning, values of free aqueous concentrations (C_{water}) for a given anaesthetic are relatively independent of temperature. For example[9], if 250 μM halothane is used (C_{water} calculated at 37 °C; see table), this will be only about 25% too high for an experiment at 20 °C. Recommended free aqueous concentrations for five inhalational agents are given in the table; these EC_{50} values are for 37 °C but are reasonable approximations for room-temperature experiments.

Estimating appropriate concentrations for intravenous agents poses different problems. These arise from their relatively rapid redistribution and metabolism, as well as from their high degree of binding to blood cells and plasma proteins. Nonetheless, reasonable estimates of free aqueous concentrations C_{water} can be obtained using blood or plasma concentrations and taking explicit account of binding. Recommended concentrations for three intravenous agents at physiological pH are given in the table.

For mammals, dose–response curves are exceptionally steep for the induction of general anaesthesia (Fig. 1), so that a concentration only 20% higher than the EC_{50} is sufficient to anaesthetize almost all animals. Moreover, at least for inhalational agents, concentrations only 2 to 4 times higher than the EC_{50} cause deleterious side effects. Thus one should treat with caution experimental data obtained at concentrations in excess of twice the EC_{50}.

EC_{50} concentrations for general anaesthesia* in mammals

Anaesthetic agent	C_{water} (μM)
Halothane†	250
Isoflurane†	320
Enflurane†	620
Methoxyflurane†	330
Chloroform†	1,000
Thiopental‡	25
Pentobarbital§	50
Propofol‖	0.4

* Lack of response to a painful stimulus.

† For the inhalational agents, C_{water} is an average value[9] for general anaesthesia in mammals.

‡ Fifty per cent of patients do not respond to a painful trapezius muscle squeeze (a stimulus shown[113] to be equivalent to a surgical incision) at a plasma[113] or serum[114] thiopental concentration of ~40 μg ml^{-1}. Using a molecular mass M_r = 241, together with a value for protein binding[113,115] of 85%, gives a free aqueous concentration of 25 μM.

§ On recovery of the righting reflex, the plasma concentration of pentobarbital in mice[116] is 15.4 μg ml^{-1}. Taking a value for plasma protein binding[117] of 61% and M_r = 225 gives a free aqueous concentration of 27 μM. But anaesthetic EC_{50} concentrations on waking or for abolishing the righting reflex are typically about half those needed to prevent movement in response to a painful stimulus[114,118], so that $C_{water} \approx 50$ μM.

‖ On waking, the blood concentration of propofol in patients[119] is ~1 μg ml^{-1}. Taking a blood/plasma partition coefficient[120] of 1.3, a value for plasma protein binding[120] of 97.8% and M_r = 178, gives a free aqueous concentration of 0.1 μM. This is comparable to values of 0.15 μM and 0.3 μM that can be calculated from corresponding data[121] for rats and dogs, respectively. Taking an average and (as above) multiplying by two gives $C_{water} \approx 0.4$ μM.

Ligand-gated ion channels

Glutamate receptors. L-Glutamate is probably the major excitatory neurotransmitter in the vertebrate CNS. Its ionotropic receptor channels (iGluRs), which mediate fast excitatory transmission, can be classified according to their selective agonists: NMDA (*N*-methyl-D-aspartate), KA (kainate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; formerly QUIS (quisqualate)). There have been surprisingly few reliable modern studies with clinical general anaesthetics on iGluRs, but a good case can be made that the dissociative agent ketamine exerts its anaesthetic effects largely by inhibiting the NMDA receptor[17,18]. Ketamine is not only relatively selective for NMDA receptors[18], but its stereoselectivity in whole animals (Box 2) is mirrored *in vitro* at the NMDA receptor[17,19]. In contrast, all iGluRs appear to be relatively insensitive to volatile agents. For example, glutamate-activated currents in dissociated neurons from the rat nucleus tractus solitarius are unaffected by 300 μM halothane or enflurane, and the most anaesthetic-sensitive iGluR responses (to QUIS) are less than 40% inhibited by 1 mM halothane[20]. Although halothane minimum alveolar concentration can be substantially reduced by selective AMPA[21] and NMDA[22] antagonists, the insensitivity of their iGluR targets argues against a major role in halothane anaesthesia. Conflicting reports exist for barbiturate inhibition of iGluRs, perhaps as a result of use-dependent block of non-NMDA receptors, as recently found[23] in cultured rat cortical neurons. In these neurons, pentobarbital was very effective at inhibiting KA currents (IC_{50} = 50 μM pentobarbital), but only after prior activation by KA. QUIS responses were more complex, with peak currents resistant but steady-state currents reduced to half by 100 μM pentobarbital. NMDA currents were very insensitive to pentobarbital[23]. Finally, it has been reported that ethanol selectively and, in common with other small aliphatic alcohols, potently inhibits NMDA receptors[24], but this selectivity has recently been questioned[25] with data showing that KA/AMPA receptors can be just as sensitive to ethanol, especially at low agonist concentrations.

Nicotinic acetylcholine receptors. The nicotinic acetylcholine receptor (nAChR) of the neuromuscular junction is the most thoroughly studied of all neurotransmitter receptors. Less is known about neuronal nAChRs, although they do share considerable sequence homologies and also function as pentameric oligomers. Work with general anaesthetics has inevitably concentrated on the more accessible muscle-type receptors. One of the few generalizations that can be made (although one probably irrelevant to anaesthesia) is that most simple anaesthetics, at high enough concentrations, are able to stabilize a desensitized form of the receptor[26]. At more relevant doses, however, other effects are observed that vary markedly between agents. Among the volatile anaesthetics, the fluorinated ethers isoflurane and enflurane are particularly effective at inhibiting the receptor[27,28], with the primary effect at the single-channel level being to reduce the mean open time[29]. Inhibition of a molluscan nAChR by isoflurane is stereoselective[28]; the *S*(+) isomer is more effective than the *R*(−) isomer, as found for general anaesthesia in mice

(Box 2). Interestingly, synaptically evoked postsynaptic currents through putative nAChRs in identified *Aplysia* neurons[27] are as sensitive to enflurane as currents evoked by ionophoretically applied ACh, suggesting that the inhibition, at least at these synapses, can be accounted for entirely by postsynaptic (rather than presynaptic) effects.

Many intravenous agents have been shown to increase the decay rate of miniature endplate currents, although rather high concentrations are required for substantial effects[30]. Other studies have shown that nAChRs can be very sensitive to some, but not all, barbiturates. For example, pentobarbital at low concentrations ($IC_{50} \approx 20\ \mu M$) reduces single channel mean open time in denervated rat skeletal muscle[31] and inhibits agonist-induced cation fluxes in *Torpedo* electroplaque membranes[32]. However, barbiturate binding to the *Torpedo* nAChR correlates poorly with anaesthetic potencies[33]. Also, the *R*(+) isomer of pentobarbital is more effective than the *S*(−) isomer[32] at inhibiting the nAChR, in contrast to their potencies in animals (Box 2). Short-chain alcohols are capable of either potentiating or inhibiting the response[34,35] to ACh (depending upon both alcohol and ACh concentrations), but long-chain alcohols are always inhibitory[34–36]. It seems likely that these effects are mediated by the alcohols binding directly to the receptor channel protein (at either excitatory or inhibitory sites), although the extent to which these alcohol-binding sites overlap with those of other anaesthetics has yet to be determined.

Although nicotinic ACh receptors are sensitive to a number of different anaesthetics, it is too early to evaluate whether or not their inhibition contributes to general anaesthesia. This should become clearer when their role in the CNS is better understood.

$GABA_A$ receptors. General anaesthetics might act by potentiating inhibitory synaptic transmission, and the $GABA_A$ receptor channel has long been considered a potential target. As with most popular ideas, however, complications and apparent contradictions appeared as work progressed, and the role of GABA (γ-aminobutyric acid) in anaesthesia has been controversial. Nonetheless, the $GABA_A$ receptor has been established as a prime anaesthetic target by recent electrophysiological studies.

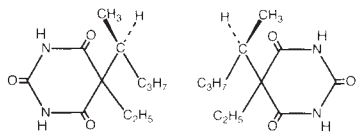
Most anaesthetics are very effective at potentiating responses to GABA. For example, 20 μM pentobarbital increases peak GABA responses by about threefold in *Xenopus* oocytes expressing whole brain messenger RNA, with the effect being accounted for by an apparent increase in receptor affinity for GABA[37]. (Pentobarbital also speeds up desensitization, but this seems simply to reflect the extent to which the channels are functionally active; that is, it is a secondary consequence of the increased affinity to GABA.) Because the major action of pentobarbital and most other anaesthetics is to shift the GABA dose–response curve to lower concentrations (Fig. 2*a*), peak Cl^- currents elicited by high levels of GABA are virtually unaffected. Effects on spontaneous inhibitory postsynaptic currents reflect more physiologically relevant interactions between anaesthetics and the postsynaptic receptor, with the decay of the currents being greatly prolonged by pentobarbital, but the rise time and peak amplitude being usually unaffected[38,39]. This behaviour is consistent with anaesthetics enhancing, and hence prolonging, receptor binding of GABA released presynaptically from vesicles in brief but high concentration pulses. Interesting, the *S*(−) enantiomer of pentobarbital is more effective than the *R*(+) isomer at enhancing GABA-induced conductance in spinal neurons[40], in line with their relative potencies in animals (Box 2).

A similar picture is emerging for other anaesthetics. The inhalational agents halothane, enflurane and isoflurane all markedly enhance currents induced by low GABA concentrations (Fig. 2*b*, *c*), but are ineffective (or even slightly inhibitory) at high GABA concentrations[20,41–43]. As with the barbiturates[37], the potentiation can be explained most simply by an anaesthetic-induced increase in the apparent affinity of GABA[20,44]. Stereoselectivity has been observed with an inhalational agent; the *S*(+) enantiomer of isoflurane, which is most potent in animals (Box 2), is about twice as effective as the *R*(−) isomer at prolonging evoked inhibitory postsynaptic currents (i.p.s.cs) mediated by $GABA_A$ receptor channels in cultured rat hippocampal neurons[45]. The residual steady-state current following desensitization by high levels of GABA is inhibited rather than enhanced by volatile agents[41], although the relevance of this to fast synaptic events is uncertain. The aliphatic alcohols appear to behave like the volatile anaesthetics[46]. In addition, the intravenous anaesthetics propofol[47] and alphaxalone[48,49] enhance the action of GABA in a variety of systems. For the steroid anaesthetic alphaxalone, the potentiation is stereoselective, with the non-anaesthetic 3-β-hydroxy-isomer, betaxalone, being ineffective[49].

Single channel studies with volatile agents[50], barbiturates[51], propofol[47] and anaesthetic steroids[52] show no changes in channel conductance, but the channel open time increases (for example, see Fig. 2*d*), consistent with the prolongation of postsynaptic currents (Fig. 2*e*). Thus the molecular basis for the potentiation of GABA action by anaesthetics is an increased open probability for the $GABA_A$ receptor channel; the extent to which this is due

BOX 2 Is general anaesthesia stereoselective?

GENERAL anaesthesia can be induced by a remarkable variety of chemical agents, including simple alcohols, alkanes, ketones, ethers and even some inert gases. Clearly no specific chemical group is required for activity. But this background of nonspecificity has tended to obscure the fact that most clinically useful agents are more complex compounds; in particular, their structures generally include an asymmetric carbon atom, so that the anaesthetic can exist in two enantiomeric forms (see figure). In practice, such agents are almost invariably administered as racemic mixtures, but when the potencies of individual optical isomers have been tested on mammals, they are stereoselective, with one of the isomers being more potent.



Stereoselectivity was first observed with the barbiturates. The optical isomers of thiopental, pentobarbital, secobarbital and hexobarbital, for example, differ in their potencies in mammals by ~2-fold[122,123]. In general, for the barbiturates, the S isomer is more potent than the *R* isomer. The two optical isomers of the dissociative anaesthetic ketamine also differ in their anaesthetic properties, with the S(+) isomer being 2 to 4 times more potent than the *R*(−) isomer in both mice[124] and humans[125]. Although with any intravenous agent it is difficult to assess accurately the extent to which pharmacokinetic factors (rates of metabolism, permeabilities, plasma protein binding, and so on) influence measured differences in potencies, the available evidence indicates that the observed stereoselectivity of intravenous agents is a real reflection of differences in their intrinsic potencies[122–124,126,127].

Optical isomers of the inhalational anaesthetic isoflurane, which are not significantly metabolized, are now available. When tested[59] for their anaesthetic effects in mice, the S(+) isomer was ~50% more potent than the *R*(−) isomer. In these experiments[59], sleep times were recorded following intraperitoneal injection, so that confirmation of this stereoselectivity will require a determination of anaesthetic EC_{50} concentrations.

The clear picture that emerges from anaesthetic potency measurements in mammals is that many general anaesthetics are stereoselective in their actions. Because the potency ratios observed with certain *in vitro* targets are comparable (see text) to those *in vivo*, anaesthetics probably exert their primary effects at a relatively small number of sites. Stereoselectivity may prove to be one of the most powerful guides as to which *in vitro* targets are relevant to anaesthesia.

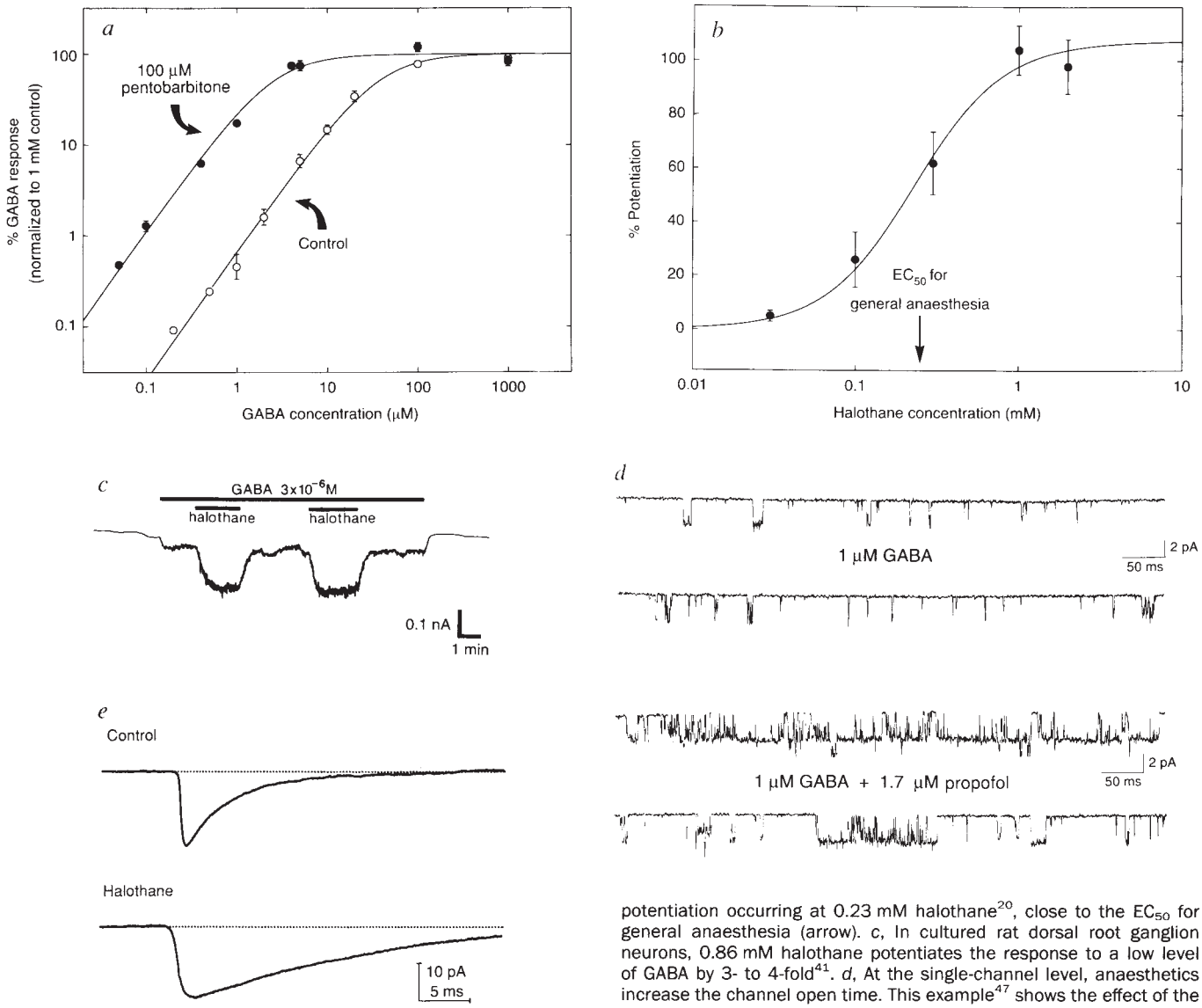


FIG. 2 General anaesthetics potentiate the actions of GABA on the $GABA_A$ receptor–channel complex. This is illustrated by recent electrophysiological results. *a*, 100 μM pentobarbital greatly enhances GABA-induced Cl^- currents in *Xenopus* oocytes injected with rat brain mRNA at low GABA concentrations but has little effect at high GABA concentrations, reflecting an increased apparent affinity of GABA for its receptor[37]. *b*, Clinically relevant concentrations of halothane potentiate responses to low levels of GABA (3 μM) in dissociated rat brain neurons, with 50% potentiation occurring at 0.23 mM halothane[20], close to the EC_{50} for general anaesthesia (arrow). *c*, In cultured rat dorsal root ganglion neurons, 0.86 mM halothane potentiates the response to a low level of GABA by 3- to 4-fold[41]. *d*, At the single-channel level, anaesthetics increase the channel open time. This example[47] shows the effect of the intravenous anaesthetic propofol on $GABA_A$ channels in bovine chromaffin cells. The probability that a channel is in a conducting state is increased by ~4-fold by 1.7 μM propofol. *e*, At a functional synapse, GABA is released presynaptically as a transient but very high concentration pulse. Under voltage-clamp conditions, the peak height of the spontaneous inhibitory postsynaptic current (i.p.s.c.) remains unchanged but the time course of the decay is prolonged by anaesthetics. This example[39] shows averages of 100 spontaneous i.p.s.cs recorded from rat hippocampal neurons in the presence and absence of 0.40 mM halothane.

to enhanced GABA binding, or to changes in channel gating, remains to be determined and may depend on the anaesthetic.

Generally speaking, almost all agents (ketamine may be an exception) at their animal half-maximal effective concentrations EC_{50} (Box 1) enhance the current induced by low levels of GABA by over 50%. (Note that a 50% increase in an inhibitory conductance can affect the membrane potential as much as a 50% inhibition of an excitatory conductance.) Does this remarkably consistent picture of potentiation of postsynaptic currents, however, translate into an enhancement of inhibitory synaptic transmission? Surprisingly little unambiguous evidence is available to answer this key question, although clear examples of synaptic potentiation (such as prolongation of evoked postsynaptic currents) have been reported[48,53]. On the other hand, some (presumably) GABAergic synapses appear to be inhibited[6,54]. Some of these discrepancies may only be apparent. For example, experimental observation of inhibitory synaptic activity often requires activation of local interneurons by excitatory synapses, which may themselves be depressed by anaesthetics. Other discrepancies are probably real. The degree of anaesthetic potentiation of $GABA_A$ receptors, for example, depends upon their subunit compositions[44], and the distribution of subunits throughout the CNS varies greatly. Indeed, the effects of anaesthetic steroids on $GABA_A$ receptors vary considerably between different brain regions[55]. In addition, anaesthetic responses may be modulated by second messenger systems, which can vary from cell to cell. The potentiating effect of ethanol apparently requires a particular γ-subunit that contains a short sequence of amino acids with a phosphorylation site for protein kinase C[56]. Also, intracellular levels of Ca^{2+} may[39] or may not[43,45] influence the anaesthetic response.

Although uncertainties remain as to the extent to which intact

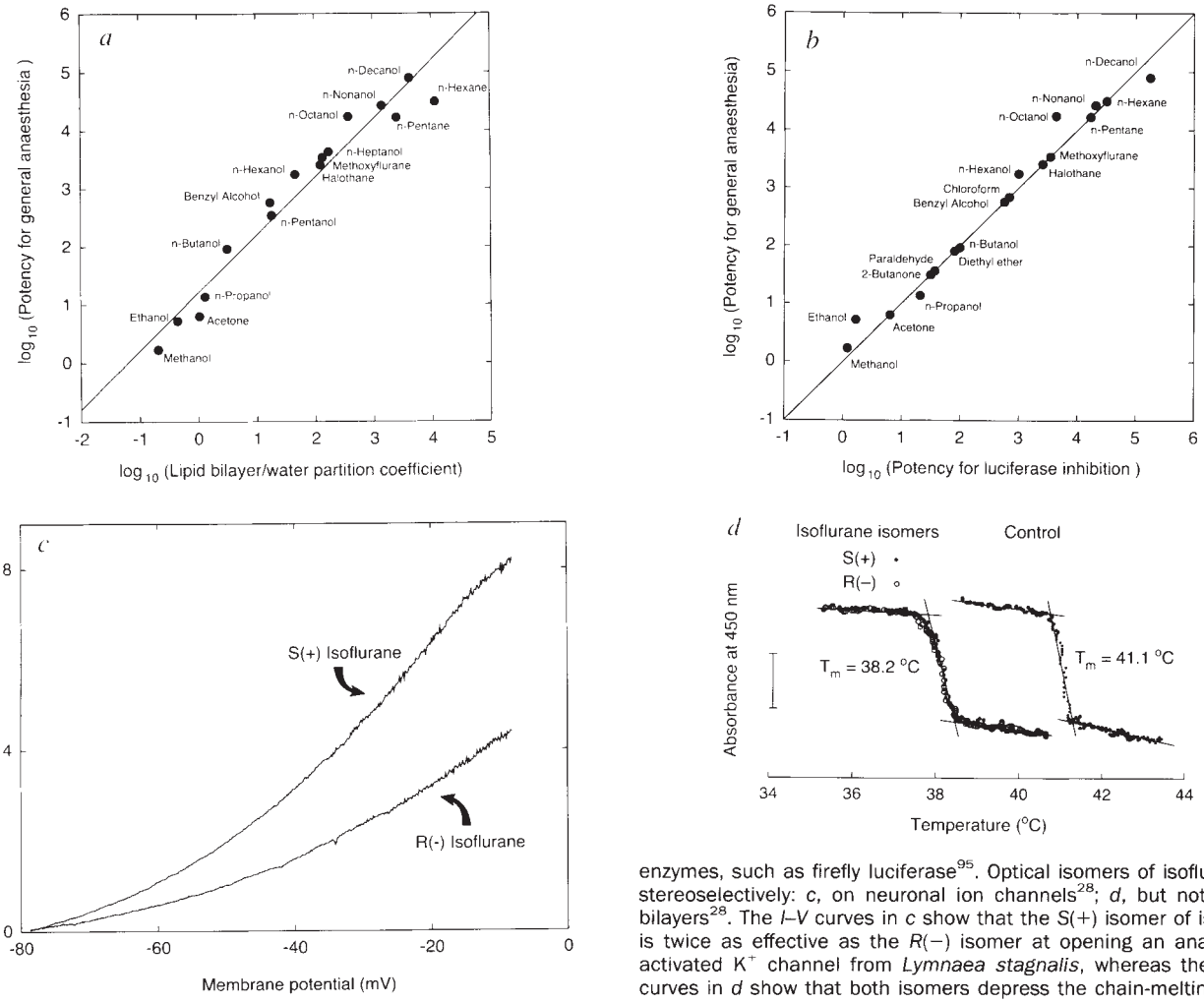


FIG. 3 General anaesthetics act by binding directly to proteins. *a*, The Meyer–Overton correlation has traditionally been interpreted as meaning that the primary target sites are lipid portions of nerve membranes[87,88]. In its modern form, shown here, a good correlation is seen to exist between the potency of an anaesthetic (reciprocal of its molar EC_{50} concentration for anaesthesia) and its lipid/water partition coefficient. *b*, General anaesthetic potencies in animals can be correlated equally well with their ability to inhibit the activity of certain soluble enzymes, such as firefly luciferase[95]. Optical isomers of isoflurane act stereoselectively: *c*, on neuronal ion channels[28]; *d*, but not on lipid bilayers[28]. The *I–V* curves in *c* show that the S(+) isomer of isoflurane is twice as effective as the *R*(−) isomer at opening an anaesthetic-activated K^+ channel from *Lymnaea stagnalis*, whereas the melting curves in *d* show that both isomers depress the chain-melting phase-transition temperature (T_m) of a pure lipid bilayer (a measure of bilayer disruption) by the same amount. The general anaesthetic potencies in *a* and *b* are taken from refs 9 and 100, or those cited in ref. 95. The lipid bilayer partition coefficients are from refs cited in ref. 87, except for the values for pentane, hexane and decanol, which were extrapolated from values for lower members of the homologous series. The luciferase inhibition data in *b* are from ref. 95; the data in *c* and *d* are from ref. 28. The line in *a* is the least-squares line of unit slope; the line in *b* is the line of identity. The calibration bar in *d* represents a change in absorbance of 0.04 units.

GABAergic synapses are functionally potentiated, there is now an impressive body of evidence that points to the $GABA_A$ receptor channel complex as a major target for most general anaesthetics.

Other inhibitory ion channels. GABA is the most important inhibitory neurotransmitter in the brain, but glycine plays a major role in the spinal cord and lower brainstem. The possible importance of these lower CNS areas, and hence of glycine, in general anaesthesia is emphasized by a recent decerebration experiment[57], which found the minimum alveolar concentration of isoflurane to be independent of cortical or forebrain structures in the rat. Nonetheless, no comprehensive anaesthetic studies have been done on the glycine receptor channel. One investigation[20] with dissociated neurons from the rat nucleus tractus solitarius found that relevant levels of volatile agents substantially potentiate inhibitory Cl^- currents activated by low but not high glycine concentrations, reflecting (as with the $GABA_A$ receptor) an apparent increase in agonist affinity. In addition, a study[47] on spinal neurons found considerable potentiation by propofol but not pentobarbital (although a fairly high glycine concentration was used).

An inhibitory synaptic K^+ current, $I_{K(An)}$, activated by low levels of volatile agents, has been found[4,28,58] in molluscan neurons. It is present in certain anaesthetic-sensitive pacemaker neurons but absent from insensitive neurons. $I_{K(An)}$ is rapidly and reversibly activated by anaesthetics, does not inactivate with time, is not voltage-gated, and responds stereoselectively to the optical isomers of isoflurane (Fig. 3*c*) with the same order of potency as general anaesthesia in mice[59]. Although these properties make it an attractive candidate for a target in general anaesthesia, it has yet to be determined whether $I_{K(An)}$ is found in mammalian neurons.

Inhibitory synapses are also attractive candidates for explaining the well-known pressure reversal of general anaesthesia. For example, the effects of pressure resemble those of strychnine[60], a glycine antagonist, and pressure reversal is not observed[61] with shrimps (which are thought not to use glycine as a neurotransmitter). As pressure almost invariably depresses synaptic transmission *in vitro*[62,63], pressure reversal could, in principle, be explained by pressure blocking inhibitory synapses, but there is now much less conviction that pressure reversal will provide fundamental clues as to how anaesthetics act. The phenomenon

of pressure reversal may prove to be incidental, rather than central, to mechanisms of general anaesthesia[63 65].

Presynaptic versus postsynaptic targets

Although it is clear that anaesthetics can have substantial effects on postsynaptic membranes, the extent to which they also act presynaptically is difficult to assess. One attractive proposal[66] was that anaesthetics might act by discharging pH gradients across the membranes of synaptic vesicles, resulting in leakage of catecholamines, but subsequent measurements[67] have shown the effects to be small. There have been many attempts to measure neurotransmitter release directly in brain slices following electrical[68] or K^+-induced[69 71] stimulation. The results vary with both preparation and neurotransmitter and often show small, but significant, changes (usually reductions) in the amount of neurotransmitter released because of the presence of anaesthetics. The multiple pathways involved in such preparations, however, make simple molecular interpretations almost impossible. An alternative approach has been to use relatively simple reflex pathways in spinal cord preparations and to disentangle pre- and postsynaptic effects by comparing evoked with spontaneous postsynaptic potentials. The most recent and careful of these studies[72,73] suggest that, at relevant concentrations of halothane and thiopental, neurotransmitter release might be inhibited by about 20%. This inhibition is most probably a result of reduced Ca^{2+} entry, rather than an effect on the exocytotic mechanisms underlying vesicle release[74]. Overall, it is likely that some anaesthetic inhibition of transmitter release occurs at many synapses, although the effects are small. However, more work is needed to establish the role presynaptic effects play in the overall anaesthetic sensitivity of synaptic transmission.

Second messenger systems

Second messengers regulate the activity of many enzymes and ion channels, sometimes by direct binding but more often by phosphorylation. One of the most important second messengers is free intracellular Ca^{2+}, and it has long been thought that anaesthetics might disrupt neuronal function by increasing its concentration ($[Ca^{2+}]_i$). With the advent of Ca^{2+}-sensitive fluorescent dyes, it is now possible to test this idea. Studies on several systems[75–79] generally show no significant sustained (as opposed to transient) effect on resting $[Ca^{2+}]_i$ at relevant anaesthetic levels and inhibition (rather than potentiation) of agonist or K^+-depolarization-induced increases in $[Ca^{2+}]_i$. The inhibitory effects have been variously ascribed to blocking of voltage-gated or receptor-operated Ca^{2+} channels, inhibition of inositol trisphosphate ($InsP_3$) production, or depletion of internal Ca^{2+} stores. Regarding $InsP_3$, which releases Ca^{2+} from internal stores, anaesthetics at relevant levels produce little[80] or no[79,81] inhibition of agonist-induced accumulation of total inositol phosphates (assumed, perhaps erroneously, to reflect $InsP_3$ levels), although substantial effects have been reported[78] for halothane in vascular smooth muscle cells. Another branch of the phosphatidylinositol second messenger pathway produces diacylglycerol, an activator of protein kinase C (PKC). The effects of anaesthetics on diacylglycerol production are not known, but its PKC target can be inhibited by anaesthetics. Although PKC inhibition seems to be small[82,83], anaesthetics inhibit a lipid-free form of the enzyme by binding to its regulatory subunit, and the anaesthetic sensitivity of lipid-bound PKC depends on the lipid composition[83]. Finally, it may be significant that liver cytochrome P450-mediated metabolism of arachidonic acid, an eicosanoid second messenger that can also be derived from inositol phospholipids, is inhibited very sensitively by a wide range of general anaesthetics[84].

Cyclic nucleotide levels have been measured in the CNS of animals killed after exposure to anaesthetics. A consistent finding is a substantial reduction in cerebellar cGMP[9], but with barbiturates this occurs at concentrations that do not reduce locomotor activity[85], suggesting that the effect may be unrelated to general anaesthesia. Most *in vitro* studies on cyclic nucleotides have used high anaesthetic concentrations, although one[81] showed that 1.25% atm. halothane at 37 °C (free aqueous concentration, $C_{water} \approx 350\ \mu M$) had no effect on basal or noradrenaline-induced cAMP accumulation in brain slices. The minimal alveolar concentration of halothane can be greatly reduced by selective α_2-adrenergic agonists[86], which, among other actions, inhibit adenylyl cyclase. However, there is no reason for believing that halothane acts on the same pathways. Indeed, it is notable that selective α_2-adrenergic antagonists, although they block the halothane-sparing actions of specific α_2-agonists, do not affect the minimal alveolar concentration of halothane on their own[86].

There is as yet little hard evidence that second messenger systems are involved in general anaesthesia. At high enough concentrations, anaesthetics can substantially affect many second messenger systems (as shown in many reports not included here), but the few studies made at relevant concentrations suggest that they may be relatively insensitive to anaesthetic perturbation.

Molecular nature of anaesthetic target sites

If general anaesthetics act by selectively targeting synaptic ion channels or the systems that regulate them, the question then arises as to how, at the molecular level, they exert their effects. Ever since Meyer and Overton discovered a correlation between anaesthetic potency and partitioning into fat-like solvents[64] (Fig. 3*a*), the traditional view has been that general anaesthetics act by disrupting the structure or dynamic properties of the lipid portions of nerve membranes[87,88]. By the early 1980s, however, accumulating evidence posed serious quantitative problems for this simple unifying idea[64,89]. Most importantly, effects on lipid bilayers at relevant anaesthetic levels were found to be implausibly small: they could generally be mimicked by temperature changes of less than 1 °C. Taken together with positive evidence for the direct involvement of proteins (see later), this has led to the almost complete abandonment of theories that postulate changes in the properties of the bulk lipid bilayer (but see ref. 90). Lipid hypotheses have subsequently been refined[91 94] and in general postulate that specialized 'domains' in membranes are not only particularly sensitive to anaesthetics, but are critical to membrane function. Probably the best formulated of these is the hypothesis that the boundary lipids surrounding membrane proteins are preferentially affected, but recent work[94] casts doubt on this idea.

Most drugs whose mechanisms are known act by binding directly to proteins. Strong evidence that general anaesthetics might also act in this way came with the demonstration[95] that the activity of a soluble lipid-free enzyme, firefly luciferase, could be inhibited by a diverse range of general anaesthetics at IC_{50} concentrations very close to animal EC_{50} values (Fig. 3*b*). These observations provided not only a viable alternative to the lipid hypotheses, but also a simple explanation for the Meyer–Overton correlation. Subsequent studies showed that this enzyme can be transformed allosterically between anaesthetic-sensitive and insensitive forms[96]. Anaesthetic-binding sites that mimic the Meyer–Overton correlation have also been found on other enzymes[83,97], whereas some proteins[98] are sensitive only to certain anaesthetics. Finally, in a recent photoaffinity labelling study[99], saturable binding of halothane ($K_d = 490\ \mu M$) to rat brain synaptosomes (presumably to proteins) has been reported.

Exceptions often prove more instructive than rules. Because both binding to proteins and dissolving in lipids can account for the Meyer Overton correlation, attention has focused on the curious lack of anaesthetic potency of long-chain compounds. In the best-studied series, the *n*-alcohols, a cut-off occurs for tadpoles after dodecanol[100] (that is, a saturated solution of tridecanol does not cause anaesthesia), and for the *n*-alkanes the cut-off in mice is after octane[101]. With lipid-free enzymes, similar cut-offs are observed[96,98,102], with the exact points of cut-off being somewhat variable. These cut-offs are most simply explained by

anaesthetics binding to protein pockets or clefts with circumscribed dimensions[102]. Explanations in terms of lipid theories have been more problematical. It was once suggested[103] that long-chain alcohols were simply not sufficiently soluble in bilayers, but this has since been shown to be incorrect[104]. More recently, it was found that whereas anaesthetic alcohols can disorder the lipids of synaptic membranes, non-anaesthetic alcohols above a certain chain-length actually increase the order; it was argued that these changes in membrane perturbation might account for the cut-off effect[105]. A prediction of this model is that compounds much larger than the cut-off should be anaesthetic antagonists. This has yet to be tested, but compounds just above the cut-off appear to be partial anaesthetics[101,106].

Probably the most definitive evidence that general anaesthetics act by binding directly to proteins comes from observations of stereoselectivity (Box 2). Even the relatively simple inhalational agent isoflurane has now been shown to act stereoselectively, not only on mammals[59] but also on neuronal ion channels[28,45] (Fig. 3*c*). Isoflurane does not, however, show stereoselective effects on pure lipid bilayers[28] (Fig. 3*d*), although additional data are needed on how the isoflurane enantiomers interact with cholesterol-containing bilayers.

Taken together, the available evidence strongly suggests that general anaesthetics act by binding directly to proteins. Why, then, are some proteins sensitive to anaesthetics whereas others (probably the majority) are not, and what is the nature of the binding sites on sensitive proteins? Some workers have argued that hydrogen bonding has a crucial role[107–110], and there is evidence that, on average, anaesthetic target sites are better hydrogen-bond acceptors than donors[111]. Whether this has any mechanistic significance is not yet clear. We think it likely that the relevant binding sites are hydrophobic pockets exposed to water rather than interfacial sites exposed to lipid hydrocarbon chains[89]. This is because the former, water-filled pockets might be expected to bind hydrophobic anaesthetics more tightly (avoiding competition from lipid hydrocarbon chains); in addition, they can more easily account for the cut-off effect[89]. There is as yet, however, no definitive evidence as to the location of anaesthetic binding sites on membrane proteins (extracellular, intracellular or transmembrane). Finally, the unusually large enthalpy changes observed when anaesthetics bind to firefly luciferase[112] may provide a clue as to what makes this protein so sensitive, perhaps reflecting anaesthetic-induced allosteric conformational changes[96] similar to those that must occur in the $GABA_A$ receptor channel.

Overview

Although general anaesthetics at high enough levels can act non-specifically on a wide variety of neuronal sites, at clinical concentrations they are much more selective and probably exert their primary effects at a relatively small number of CNS targets. These are most likely to be postsynaptic ligand-gated ion channels. Some agents act predominantly at excitatory receptors (such as ketamine at NMDA receptors), but anaesthetic potentiation of inhibitory synaptic receptors (mainly $GABA_A$) best matches the pharmacological profile of a wide variety of agents for producing general anaesthesia in mammals. Some uncertainty remains, however, over the extent to which the activity of intact inhibitory synapses is potentiated by anaesthetics. In addition, some agents (pentobarbital for example) are clearly effective at both inhibitory and excitatory postsynaptic receptors, so the balance between the inhibition of excitatory synapses and the potentiation of inhibitory synapses in causing general anaesthesia still needs to be established.

Although much attention has focused on voltage-gated ion channels, as a class they are very resistant to clinical concentrations of general anaesthetics. Some voltage-gated Ca^{2+} channels are somewhat more sensitive, and their inhibition may underlie the small reduction of neurotransmitter release that occurs at some synapses.

At the cellular level, anaesthetic actions vary from neuron to neuron owing to the presence of differing ion channels and ion-channel subunits, as well as second messenger regulatory systems. But the case for any major involvement of second messenger systems in general anaesthesia is unproven; the few studies at pharmacologically relevant concentrations indicate that they may be relatively insensitive to anaesthetics, but further investigation should provide more definitive evidence.

At the molecular level, anaesthetics almost certainly act by binding directly to proteins rather than by perturbing lipid bilayers. Anaesthetics probably influence protein activity by binding to pockets or clefts and causing small changes in protein conformation. □

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ACKNOWLEDGEMENTS. We thank R. Dickinson and A. C. Hall for their comments on the manuscript, C. J. Lingle for the Ca^{2+} channel data in Fig. 1, and N. Akaike, J. J. Lambert, M. B. MacIver, T. Narahashi and I. Parker for the data in Fig. 2. This work was supported by the MRC, the NIH, Anaquest and the BOC Group Inc.

Atomic model of plant light-harvesting complex by electron crystallography

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The structure of the light-harvesting chlorophyll *a/b*–protein complex, an integral membrane protein, has been determined at 3.4 Å resolution by electron crystallography of two-dimensional crystals. Two of the three membrane-spanning α-helices are held together by ion pairs formed by charged residues that also serve as chlorophyll ligands. In the centre of the complex, chlorophyll *a* is in close contact with chlorophyll *b* for rapid energy transfer, and with two carotenoids that prevent the formation of toxic singlet oxygen.

PHOTOSYNTHESIS in all green plants uses solar energy collected by the light-harvesting chlorophyll *a/b*–protein complex associated with photosystem II (LHC-II). LHC-II is the most abundant membrane protein in chloroplasts, accounting for half of the pigments involved in plant photosynthesis. Each polypeptide of 232 amino acids[1,2] binds and organizes a minimum of 12 chlorophylls and 2 carotenoids (xanthophylls) for light-harvesting and energy transfer to the reaction centres of photosystems I and II.

The molecular mechanisms underlying these processes can be understood only if the structures of the participating pigment–protein complexes in the photosynthetic membrane are known in detail. The structure of a bacterial photosynthetic reaction centre has been solved at high resolution[3,4], and that of a cyanobacterial photosystem I complex has been solved at 6 Å (ref. 5). So far, the most detailed structure of a membrane antenna complex has been a 6 Å map of LHC-II (ref. 6). Here we present an atomic model of LHC-II based on a three-dimensional map at 3.4 Å resolution.

High-resolution electron microscopy

Detergent-solubilized, purified LHC-II forms highly ordered two-dimensional crystals[7,8], which are ideal objects for electron crystallography. Electron diffraction of two-dimensional crystals yielded the structure factor amplitudes[9]. Phases were determined directly by processing[10–12] electron micrographs of tilted two-dimensional crystals. Images were recorded with an electron cryomicroscope capable of resolving 2.0 Å object features at a specimen-stage temperature of 4.2 K (ref. 13). At very low temperature the effects of radiation damage are much less severe than at room temperature. More electrons can therefore be used to take an image, so that high-resolution phases can be determined more accurately.