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Multimodal Anesthesia and Systems Neuroscience

The New Frontier

MECHANISTIC research is entering an exciting era in which knowledge about the molecular targets for general anesthetics is beginning to influence clinical practice. Identifying the receptors and neuronal circuits that underlie the neurobehavioral effects of anesthetics is particularly important if we are to develop new anesthetic agents and advance beyond the "ether era." The clinical properties of contemporary inhaled anesthetics are considerably more desirable than ether; nevertheless, these halogenated hydrocarbons have an extraordinarily low therapeutic index and cause widespread neurodepression.

The anesthetic state encompasses several behavioral endpoints, including loss of consciousness, amnesia, immobility, and analgesia. The current clinical practice of "balanced anesthesia" depends on a combination of drugs that are relatively nonselective to produce these behavioral endpoints. A popular working hypothesis is that the next generation of general anesthetics will be based on a multimodal strategy, whereby patients will receive drugs that target highly specific receptor populations and neuronal networks to produce the desired behaviors. In this context, the article by Vanini *et al.*[1] is important because it identifies a region of the brain stem that contributes to the loss of consciousness produced by the anesthetic isoflurane. The authors used microdialysis to measure levels of γ-aminobutyric acid (GABA) in the pontine reticular formation of cats, during wakefulness and isoflurane anesthesia. GABA levels decreased during anesthesia, and the power of the electroencephalogram and activity of the electromyogram covaried with the GABA levels.

They also found that injection of a GABA reuptake inhibitor, nipecotic acid, into the pontine reticular formation of rats increased isoflurane induction time, whereas injection of a GABA synthesis inhibitor, 3-mercaptopropionic acid, had the opposite effect. From these results, they conclude that isoflurane anesthesia is due, at least in part, to a decrease of GABA levels in the pontine reticular formation.

This Editorial View accompanies the following article: Vanini G, Watson CJ, Lydic R, Baghdoyan HA: γ-Aminobutyric acid–mediated neurotransmission in the pontine reticular formation modulates hypnosis, immobility, and breathing during isoflurane anesthesia. ANESTHESIOLOGY 2008; 109:978–88.

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The authors are to be commended because, despite the accumulation of much information over the past several decades about anesthetic-sensitive receptors, few researchers have attempted to situate this information in the context of how the central nervous system operates at the systems level. Systems neuroscience is the field of science research that studies the function of neuronal circuits and connectivity relations between neuronal networks with *in vivo* models in intact subjects. It has been proposed that a systems approach is essential for contemporary drug discovery and development.[2] Nevertheless, only recently have attempts been made to determine which neuronal networks are augmented or depressed, the anesthetic dosages at which these effects occur, and how such effects result in behavioral phenomena.[3–6] To achieve the goal of multimodal anesthesia, we must identify, at the systems neuroscience level, the anesthetic actions that are necessary and sufficient to produce the desired behavioral end points. This task is extraordinarily challenging because of the complexity of human neurophysiology and anesthetic pharmacology. Although such complexity is daunting, it can be exploited for therapeutic gain.[2]

To appreciate the level of complexity and illustrate why we need to understand network relations and connectivity, let us consider the effects that anesthetics have on a single neurotransmitter system. GABA is the major inhibitory transmitter in the central nervous system. The $GABA_A$ subtype of receptor is a chloride-permeable ion channel that mediates the majority of GABAergic actions. $GABA_A$ receptors are ubiquitous in the central nervous system, and most general anesthetics increase their activity, causing an increase in membrane permeability to chloride and an influx of negatively charged ions.[7] This results in membrane hyperpolarization, the shunting of excitatory input, and depression of neuronal firing. Most inhaled and injectable anesthetics have at least three distinct concentration-dependent actions on $GABA_A$ receptors: They increase the potency of endogenous GABA, they modify receptor desensitization, and they directly activate the channel in the absence of GABA.[8] The $GABA_A$ receptor is a heteropentameric complex made up of subunits encoded by at least 19 genes.[9] The subunit composition of the $GABA_A$ receptors dramatically alters the potency of GABA and the pharmacology of the receptors, most notably with regard to the effectiveness of general anesthetics.[7] The distribution of subunits varies considerably between brain region, the neuronal population within a region, and even the subcellular domains of a single

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neuron. Given that an increase in $GABA_A$ receptor–mediated neurotransmission typically causes neurodepression, what might account for the observation by Vanini *et al.* that an isoflurane-induced decrease in GABA levels in the reticular pontine formation contributes to the loss of consciousness?

The reticular formation is part of a complex circuit in the brain stem whose role in regulating sleep and wakefulness we are only now beginning to dissect. This part of the brain contains neurons that contribute to the ascending arousal pathways, from the noradrenergic locus coeruleus, the cholinergic pedunculopontine, the laterodorsal tegmental nuclei, and others (for review, see Saper *et al.*[10]). There have been hints over the years that GABAergic neurons in this region contribute to the regulation of these cell groups, although some of these seem contradictory. For example, injections of the GABA agonist muscimol into the pontine reticular formation in rats caused increased wakefulness and increased latency to rapid eye movement (REM) sleep, whereas the GABA antagonist bicuculline had the opposite effect.[11] Similar results were reported when GABA agonists were injected into the rostral pontine reticular formation, ventral to the locus coeruleus in cats.[12] However, other researchers have reported that injections of muscimol into the brain stem in the mesopontine tegmentum of rats caused an anesthesia-like state, with loss of consciousness.[13]

These seemingly contradictory results can be explained by a careful review of the studies and a consideration of the injection sites and neuronal circuitry. The injection sites in the mesopontine tegmentum[13] were approximately 1–2 mm more rostral (toward the front or nasal region) than the sites used by other researchers (fig. 1).[11,12] This region of the brain stem contains GABAergic neurons that are involved in regulating REM sleep, but many of these neurons are GABAergic REM-off cells, meaning that their firing *prevents* transitions into REM sleep. Lesion of these GABAergic REM-off cells,[14] or injecting the area with muscimol to inhibit the neurons,[15] increases REM sleep.

The REM-off neurons located at the junction of the midbrain and the pons appear to target nearby GABAergic neurons in the rostral pontine reticular formation. These in turn project back to the GABAergic neurons a millimeter rostral, at the mesopontine tegmentum.[14,16] This mutually inhibitory pattern of GABA–GABA interactions forms what electrical engineers would call a "flip-flop" switch. A flip-flop electrical circuit is one that changes between two states when current is applied (*i.e.*, changes from 1 to 0 or 0 to 1). In this type of switch in neuronal networks, each side inhibits the activity of the other to provide rapid and complete transitions, as observed when animals drop into or out of REM sleep within 1 or 2 s. Excitatory glutamatergic neurons are mixed in with the GABAergic ones, providing long outputs to the brain stem and the basal forebrain. Rostral neurons, mixed in with the REM-off cells, reinforce motor tone and wakefulness, whereas more caudal neurons, mixed in with the REM-off population, activate the phenomena associated with REM sleep.[14]

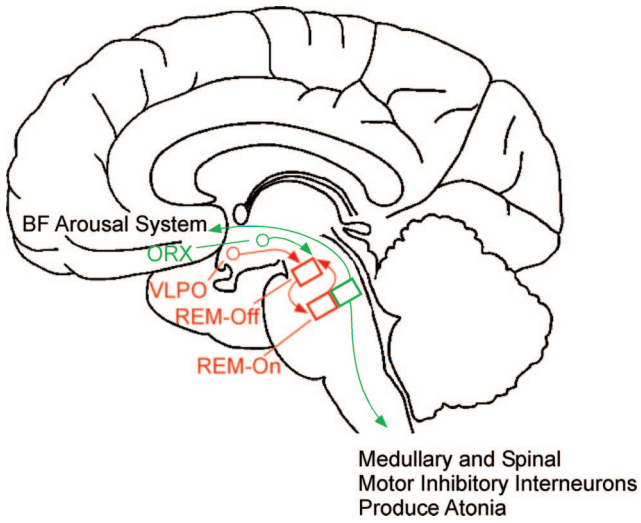

Fig. 1. A drawing of the upper brainstem and basal forebrain (BF), indicating the location of the arousal system, which includes the rapid eye movement (REM) sleep "flip-flop" switch in the pons, a key site at which γ-aminobutyric acid (GABA) is thought to influence wake–sleep states. GABAergic neurons in the mesopontine tegmentum (*red squares*) are REM-off neurons (which fire when the brain is not in an REM state). These neurons inhibit GABAergic REM-on neurons (*red squares*) at the rostral pontine level (which fire during REM sleep). The REM-on neurons in turn inhibit the REM-off population, and this mutual antagonism produces a flip-flop switch in which the two sides are mutually inhibitory. This results in rapid and complete transitions between states. Glutamatergic neurons (*green arrows*) are also found in the REM-on zone, and some of these send ascending outputs to the forebrain to cause electroencephalographic desynchronization seen in both arousal and REM sleep. Other glutamatergic neurons send axons to the lower brainstem, where they control eye movements and muscle tone. Orexin (ORX) neurons excite the REM-off neurons (thus preventing REM sleep). Ventrolateral preoptic (VLPO) neurons promote REM sleep by inhibiting the REM-off neurons. Injections of GABA agonists into the mesopontine tegmentum inhibited the REM-off neurons and caused REM sleep. However, injections of GABA agonists just 1 mm caudally in the rostral pontine tegmentum had the opposite effect.

The actions of GABA in regulating wakefulness and sleep critically depend on the location in the brain stem being investigated. A particular neurotransmitter may serve one type of activity at one site and have exactly the opposite effect 1–2 mm away, where it is used by a counter-posed circuit. The work of Vanini *et al.* clearly confirms that GABA increases wakefulness, possibly by suppressing mechanisms that lead to increased REM sleep.

Interestingly, isoflurane, which usually enhances $GABA_A$ receptor–mediated neurotransmission, decreases GABA release at this pontine site.[1] This could result from isoflurane depressing the firing of cells that release GABA into the pontine reticular formation, thus augmenting the ability of sleep-promoting or REM-on circuitry to inhibit

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conscious wakefulness. This finding is consistent with recent studies from our laboratory (C.B.S.) on the patterns of c-Fos expression in the brain during anesthesia produced by isoflurane and other anesthetics.[4] In general, we have found that the expression of c-Fos is augmented in the sleep-active ventrolateral preoptic nucleus (which itself is GABAergic), and in the locus coeruleus. The latter finding was surprising, given that anesthesia is expected to inhibit the arousal circuitry. However, the sleep-active locus coeruleus also contributes to descending antinociceptive pathways, which form an important component of anesthesia. Lesions of the locus coeruleus impair the analgesia associated with GABAergic anesthetics.[4] Therefore, the effects of anesthetic drugs on wake–sleep circuitry may be complex, and ultimately surprising.

Flip-flop switches, crucial relay stations, or decisive nodes that control information flow and the architecture of critical pathways in the central nervous system may be precisely the targets that can be harnessed for multimodal anesthesia. For example, if the subunit composition of the $GABA_A$ receptors that inhibit arousal differs from that of receptors that activate sleep-active neurons, subunit-selective drugs could produce a swift transition to unconsciousness. Such an on–off switch for consciousness could be coupled with anesthetic-sensitive switch systems regulating the information-processing circuitry, such as those in the hippocampus (to produce amnesia) and the thalamocortical pathways (to modify sensory processing). Without doubt, there are multiple mechanisms operating simultaneously in many different brain regions to produce the anesthetic state. The current challenge is to design experiments that allow us to determine which of these mechanisms are essential. The article by Vanini *et al.* should be viewed as a call to arms, encouraging us to work out, at a systems level, the brain circuitry that underlies the anesthetic state. This type of systems research requires integrative thinking and offers an opportunity for breakthrough advances. The next few years of work in this field should be very exciting indeed.

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