■ REPORT OF A SCIENTIFIC MEETING

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International Workshop on Anesthetic Mechanisms. Takamatsu, Japan, December 12–14, 1994.

The International Workshop on Anesthetic Mechanisms, December 12–14, 1994, was held in Takamatsu, a harbor town on the smallest of Japan's four main islands, Shikoku. The meeting was organized by Professor Kenji Ogli (Kagawa Medical School) and Professor Issaku Ueda (University of Utah), with the aid of a committee comprised of representatives from about 20 Japanese medical schools and universities. Meeting sessions were held on all 3 days and consisted mostly of serial 25-min presentations by invited principal speakers. Sessions were devoted to membranes, receptors, genetics, and macromolecules, as well as physicochemical properties and neurophysiology, and followed by panel discussions. Also included were an evening roundtable discussion, a plenary lecture, and poster presentations.

After a brief welcome by Ogli, Dr. Rod Eckenhoff (University of Pennsylvania) reviewed the principles of photoaffinity labeling using halothane and findings made with both biomembranes and pure model proteins (principally, albumin). Both systems yielded data supporting specific, saturable labeling of proteins. In addition, his results suggested that halothane binds preferentially to protein/lipid interfaces. Dr. Nathan James (Jefferson) explained how drug partitioning among different lipid domains might account for some effects of alcohol on cell membranes. Dr. Elizabeth Rowe (University of Kansas) discussed titration calorimetry experiments designed to assess the importance of lipid composition on the partitioning of alcohols into vesicles. Cholesterol content seems to be more important than the type of phospholipid. The role of enthalpy (thermodynamic heat content) was greater than expected, indicating that the current models for anesthetic partitioning into membranes are overly simplistic. Monolayer systems consisting of dipalmitoyl phosphatidyl choline (DPPC) and fluorocarbon fatty acids were presented by Dr. Shigekazu Yamamoto (Fukuoka University). The physical properties of such monolayers are acutely sensitive to the structure of the fatty acid. Fluorocarbon fatty acids interact more strongly with DPPC than do hydrocarbon fatty acids. Dr. Satoru Iiyama (Kinki University) related changes in the surface potential of synthetic lipid membranes by local anesthetics to their effects on excitable membranes. His collaborator, Dr. Yukio Suezaki, presented calorimetric evidence that perturbations of synthetic lipid membranes so typical of anesthetics may occur with other hydrophobic, *nonanesthetic* drugs, such as diltiazem.

The session on receptors was begun by Dr. Jim Dilger (State University of New York at Stony Brook), who discussed inhibitory and potentiating effects of anesthetics on nicotinic acetylcholine receptor (nAChR) channels. The different patterns of single channel activity induced by ether, isoflurane, and propofol were interpreted in terms of a single inhibitory (channel-blocking) model. Differences in drug dissociation rates account for the different patterns. Dr. Howard Wang (University of California at Santa Cruz) studied the interactions between halothane and ^{3}H-phencyclidine (PCP), a noncompetitive inhibitor of the nAChR. He showed that low concentrations of halothane increase the dissociation of PCP, whereas high concentrations competitively inhibit PCP binding. One interpretation is that halothane has two binding sites: one close to the PCP binding site and a second at the PCP binding site. The focus then shifted to the other major ligand-gated ion channel model, the $GABA_A$ receptor ($GABA_A$-R). Dr. Toshio Narahashi (Northwestern University) has studied both rat dorsal root ganglion neurons in primary cell culture, and human embryonic kidney (HEK)-293 cells transiently expressing various rat $GABA_A$-R subtypes, using whole cell patch clamping. He clearly demonstrated that, although ethanol's effects on peak current are subtype-independent ($\alpha_1\beta_2\gamma_{2S}$ responses equivalent to those for $\alpha_1\beta_2\gamma_{2L}$), its effects on desensitization are highly subtype-dependent (α_6 is required). Dr. Jay Yeh (Northwestern University) then presented whole cell patch clamp observations of recombinant rat $GABA_A$-Rs expressed in HEK-293 cells. He observed that halothane exerted a similar dual action on these recombinant channels. At low GABA concentrations (relative to the respective K_D), halothane *potentiated* the chloride current with Hill coefficients of approximately 2. At high GABA concentrations, halothane *inhibited* peak current and further accelerated desensitization. These data were interpreted as supporting a model in which anesthetic effects are a function of the conformational state of the receptor/channel. Next, Dr. Yoshimi Ikemoto (Fukuoka University) discussed the effects of propofol on dissociated rat hippocampal pyramidal neurons. As in many other systems, propofol potentiates GABA-induced current at therapeutically relevant concentrations, but at slightly higher concentrations, propofol activates a bicuculline-inhibitable inward chloride current.

Dr. Phil Morgan (Case Western Reserve University) began the genetics session by explaining the advantages of the *Caenorhabditis elegans* genetic model, and the progress toward identifying (mapping) genes involved in its anesthesia responses. Recently identified mutations appear to fall into two categories: one affecting responses to enflurane, isoflurane, and ether; the other affecting responses to enflurane and isoflurane only. Based on this, as well as responses of double mutants, a hierarchical model of the genes (products) controlling volatile anesthetic sensitivity was proposed. Dr. Len Firestone (University of Pittsburgh) then presented evidence that mice selectively bred for sensitivity or resistance to diazepam are cross-sensitive/-resistant to volatile anesthetics. A molecular mechanism for this may be related to his findings that $GABA_A$-Rs derived from the brains of these mice had sensitivity or resistance to halothane *in vitro* corresponding to that found *in vivo*. The most relevant $GABA_A$-R subtypes will be determined in genetically engineered mice with appropriate targeted disruptions. Dr. Sumiko Gamo (University of Osaka Prefecture), the final speaker of this session, reviewed her discovery of several ether-resistant, -sensitive, and -hypersensitive *Drosophila* mutants. Attempts to identify and clone the relevant gene(s) have involved the transposon-tagging method, which uses a readily identifiable fragment of DNA to tag a specific genetic locus. Genes labeled by this method were found to be expressed mostly in the central and peripheral nervous system of larvae and adults. At least five genomic loci were tagged, one of which corresponded to an area close to the *para* locus, known to encode for a sodium channel protein.

The evening roundtable discussion, "Specific of Nonspecific?" featured a debate between Dr. Nick Franks (Imperial College) and Ueda. Franks reviewed how pharmacologic tools such as the well known potency cutoff among homologous alkanols, stereoselectivity, and temperature-dependence of anesthesia, all lead to the conclusion that anesthetics interact directly with proteins. Ueda employed data

derived from differential scanning calorimetry and Fourier transform infrared spectroscopy to support his view that the observed interactions of anesthetics with proteins can be explained by nonspecific protein unfolding ("conformational entropy"). (This should be distinguished from "nonspecific" *lipid* perturbation theories). As expected, these alternative views sparked a heated discussion about the relevance of particular *in vitro* models and the philosophical obstacles to disproving the entropy hypothesis.

Dr. Danuta Kosk-Kosicka (Johns Hopkins University) began the macromolecule session by demonstrating the sensitivity of the erythrocyte Ca^{++}-ATPase to general anesthetics. Calmodulin-stimulated activity is particularly sensitive, and importantly, this is true whether the enzyme is membrane-bound or solubilized, supporting the importance of direct protein interactions. Dr. Jim Trudell (Stanford University) emphasized that most lipid perturbation hypotheses cannot explain how lipid effects are coupled to proteins. The polymixin/lipid model system is an exception. Polymixin causes a lipid phase separation, and this is sensitive to anesthetics. The results indicate that the partitioning and the action of anesthetics varies with different lipid domains. Next, Dr. Alex Evers (Washington University) discussed NMR and photoaffinity labeling experiments on anesthetic interactions with water-soluble proteins such as fatty acid binding proteins and luciferase. Results indicate that halothane binds to saturable sites on these proteins and that binding involves considerable specificity, because affinity can be altered by a single amino acid substitution. However, different anesthetics may not bind to a common site. Dr. Akira Shibata (University of Tokushima) then presented a circular dichroism study of the effects of volatile general anesthetics on firefly luciferase. The ability of these agents to unfold the protein and inhibit bioluminescence was reasonably well correlated with their anesthetic potency *in vivo*, supporting a "nonspecific" but protein-based mechanism of action. Phospholipase A_2 (PLA_2), a protein that hydrolyzes lipids with both (+) and (−) changes at physiologic *p*H, was the focus of Dr. Rodney Biltonin's (University of Virginia) presentation. Anesthetics such as dibucaine and octanol have little effect on hydrolysis of lipid monomers by PLA_2 or on the binding of PLA_2 to lipid vesicles. However, anesthetics alter the rate of PLA_2 hydrolysis of the lipids in a bilayer. Lateral phase separation and membrane curvature may be involved in these interactions. Dr. Bill Lieb (Imperial College) reviewed the recent data from his laboratory on effects of isoflurane stereoisomers. The partitioning of isoflurane into lipid bilayers is not stereoselective. Ion channels that are particularly sensitive to anesthetics (*e.g.*, $GABA_A$-R from rat cerebellar neurons) exhibit stereoselectivity; those with low sensitivity (*e.g.*, a *Limnea* K^+ conductance) are not stereoselective. These results suggest that stereoselectivity may be useful to distinguish relevant anesthesia effects from epiphenomena. Anesthetic effects on volume-sensitive ion transporters, such as the swelling-activated KCl cotransporter, were presented by Dr. George Kracke (University of Missouri). In erythrocytes, volatile agents inhibited Na^+/H^+ exchange while stimulating KCl cotransport. This is consistent with the notion that anesthetics alter the volume set point of the cell, perhaps through some membrane perturbation. Dr. Toshiaki Hamanaka (Osaka University) concluded this session by correlating structural (x-ray diffraction and absorption spectroscopy) and functional (proton-pumping) data from bacteriorhodopsin in the purple membrane of *Halobacterium balobium*. At low anesthetic concentration, pumping is enhanced by a relatively short-lived crystalling specie; at high concentrations, pumping is blocked by a long-lived noncrystal. X-ray diffraction studies with diiodomethane were consistent with binding at an interface between the lipid and protein and/or membrane and water. With this final bit of evidence, the macromolecular session could be said to feature precedents for every conceivable sort of molecular interaction with anesthesia: specific and nonspecific interactions with proteins, lipid perturbations, and lipid-protein interface interactions.

The plenary lecture was delivered by Dr. Ichiji Tasaki, who retired from the National Institutes of Health Laboratory of Cell Biology after a distinguished career spanning half a century and two distant nations. He recounted his long-term fascination with the finding many years ago that nerve depolarization is accompanied by local swelling that is inhibited by local anesthesia. Proposed mechanisms included Na^+/Ca^{++} exchange and lipid structural transitions. After an afternoon poster session, a banquet in honor of Tasaki's, Ogli's, and Ueda's many years of contribution to this field concluded the second day.

The physicochemical properties session began with Dr. Don Koblin's (University of California, San Francisco) presentation featuring a number of perfluoroalkanes with significant lipid solubility but little or no anesthetic potency (in defiance of the Meyer-Overton rule). Clearly, these agents should prove useful to evaluate the relevance of models of the anesthetic site. Dr. Camille Sandorfy (University of Montreal) focused on the possible role of acidic hydrogens in forming weak hydrogen bonds between anesthetics and membrane constituents. Dr. Mitsuhiro Takasaki (Saga University) analyzed the thermodynamic changes that occur when general anesthetics dissolve in aqueous media. Dissolving any of the inhaled anesthetics was exothermic (negative enthalpy), but entropy fell as well, consistent with formation of hydration layers. Moreover, these two parameters were highly correlated, despite marked structural dissimilarities among the anesthetics, supporting that effects on water may yet be important to anesthetic potency. Dr. Shoji Kaneshima (University of Tokushima) described the effects of volatile anesthetics and hydrostatic pressure on the phase transition behavior of lipid vesicles. Partition coefficients were estimated for each lipid phase, based on the depression of transition temperature; pressure raised transition temperature and thus seemed to "squeeze out" the anesthetics. Dr. Alister Macdonald (Aberdeen University) then broadened the scope of the pressure discussion with patch-clamp studies of anesthetic and hyperbaric helium effects on an insect muscle glutamate-gated channel. Whereas all of the effects on channel kinetics of 1.5 atmospheres absolute (ATA) N_2O could be reversed by 100 ATA of helium pressure, only the mean open time effects of ketamine were so reversible. These data indicated the specificity with which both anesthetics and pressure may act and are not easily reconciled with a unitary lipid-based hypothesis. Dr. Seiji Sawamura (Ritsumeikan University) used infrared spectroscopy to study the effects of pressure and halothane on DPPC structure. His data indicated that halothane promotes hydrogen-bonding, and this is reversed by high pressure. Dr. Katsuhiro Tamura (University of Tokushima) closed the session by discussing experiments with yeast whereby pressure is used to reverse ethanol's inhibition of growth. Alone, pressure exerted toxic effects, but other data indicated that it was able to antagonize ethanol's growth-inhibiting properties. Additional studies suggest that prior ethanol exposure protects yeast against stresses such as hyperthermia and hyperbaric pressure, perhaps by induction of heat-shock proteins.

The final session, neurophysiology, was begun by Dr. Tony Angel (University of Sheffield). Using an elegant rat model for tracking the rostral transfer of somatosensory information under anesthesia, several subclasses of anesthetic agents were identified. All agents increased the latency of the evoked somatosensory cortical response. The inhalation agents typically attenuated subcortical (thalamic) signals as

well. Etomidate and propofol did not have much effect on the thalamic signal. Benzodiazepines stabilized the discharge of cells at the thalamic and cortical levels. Such data favor a multisite (in the anatomic sense) interpretation for anesthetic action. Franks is well known for his view that, however many sites there are, hydrophobic pockets in proteins comprise the most plausible site(s) of action for general anesthetics. He emphasized the difference between the temperature dependence of anesthetic solubility when in the gas *versus* aqueous phases and demonstrated how misunderstanding this difference often confounds published results. Specifically, in contrast to the steep temperature dependence of solubility in the gas phase (usually necessitating compensatory calculations), the slope for the aqueous phase is comparatively flat (thus constant). Then, using temperature dependence as a probe, Arrhenius relationships were presented for inhibition of neuronal nicotinic currents and luciferase activity. The slopes indicated that anesthetic apparent binding enthalpies were far more negative than those observed for binding to lipids. Could this explain why some sites seem so much more sensitive to anesthetic effects than others? The focus of Dr. Joan Kendig's (Stanford University) presentation was the neonatal rat spinal cord, which provides a compact, well defined model for most of the important synaptic (receptor) types (*e.g.*, glutamate, AMPA/kainate, NMDA, $GABA_A$, and metabotropic receptors). All anesthetics were noted to depress transmission between primary afferent sensory neurons and ventral motor neurons. However, each agent manifested a unique profile of effects on these receptor-specific pathways, suggesting multiple sites and, perhaps, mechanisms for anesthesia. Dr. Sid Simon (Duke University) has studied sensory systems at the cellular level, particularly the physiology of taste, to deduce the site(s) of anesthetic alcohol actions. Menthol is one such alcohol that is known to have a receptor on sensory (cold) fibers. Interestingly, whereas menthol activates this site, hexanol inhibits it, although at supraphysiologic concentrations. The session concluded with Dr. Sheldon Roth's (University of Calgary) presentation about anesthetic effects on rhythmic slow wave activity (RSA) in carbachol-stimulated perfused rat hippocampus *in vitro*. The volatile agents reversibly altered total power, amplitude, peak frequency, and burst length of RSA, but differential effects were observed in the hippocampal sublayers of dentate granule and CA1 pyramidal cells. The data were interpreted to support multiple anesthetic mechanisms at selective cellular sites, which might be said to summarize the views of the majority of the meeting's principal speakers. Ueda closed the presentations by underscoring this "multiple sites" viewpoint and urged greater communication between the physical chemists and biologists, as well as between Eastern and Western scientists. (If the intensity of the hallway meetings and furious swapping of business cards and electronic mail addresses during the meeting were any indication, this process was already well underway!)

The workshop was concluded at a dinner honoring the exceptionally fine work of the meeting organizers from Kagawa Medical School, most notably, Dr. Satoshi Yokono, Dr. Ikuko Tsukamoto, Dr. Junko Nogaya, and their coworkers. Incidentally, this is the same energetic team involved in producing the new international journal *Progress in Anesthetic Mechanism*.

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