

## *When You Breathe IN You Inspire, When You DON'T Breathe, You . . . Expire*

### *New Insights Regarding Opioid-induced Ventilatory Depression*

THE sedative, analgesic, and euphoric effects of opioids have been known since antiquity. First described by the Sumerians some 6,000 yr ago, the euphoric and analgesic properties of the seed-pod exudate of *papaver somniferum* are described in Homer's *Iliad* and were well known to physicians by the time of Hippocrates (460–377 B.C.E.). However, since the time of Pliny the Elder (23–79 C.E.), it has also been known that opioids may produce life-threatening respiratory depression, which limits both their utility and their safety. This issue of ANESTHESIOLOGY includes two reports that enhance our understanding of these respiratory depressant effects. Bouillon *et al.* at Stanford provide new insights into the acute effect of rapidly acting opioids on ventilatory control through the use of a new modeling technique, which permits non-steady state estimates of the interaction between the respiratory depressant effect of remifentanyl and the respiratory stimulating effect of retained carbon dioxide.<sup>1</sup> In contrast, Romberg *et al.* at Leiden studied the pharmacodynamics of morphine-6-glucuronide (M6G)<sup>2</sup>; this is of particular interest because of the possibility that M6G may have greater affinity for  $\mu_1$  opioid receptors (responsible for analgesia) than for  $\mu_2$  receptors (responsible for respiratory depression), offering the promise of systemic analgesia with an increased margin of safety.<sup>3</sup>

There are several ways to study the effects of sedative, analgesic, and anesthetic drugs on the ventilatory response to carbon dioxide. Perhaps the simplest is to follow changes in respiration (rate, tidal volume, minute ventilation) and carbon dioxide tension (arterial, end-tidal) during room air breathing. This type of closed-loop design (so called because carbon dioxide tensions are regulated by the body's intrinsic negative feedback system) most closely mimics the clinical situation where

patients breathe spontaneously after receiving sedative medications. However, measurements based on resting ventilation have significant disadvantages that make it difficult to draw meaningful pharmacodynamic conclusions. First, resting ventilation and carbon dioxide tension are determined by the intersection of the carbon dioxide ventilatory response curve (fig. 1, *curve A*) and the carbon dioxide excretion (metabolic) hyperbola (fig. 1, *curve C*). A drug that causes a 50% decrease in the slope of the carbon dioxide response (fig. 1, *curve B*), would cause  $P_{aCO_2}$  to increase by less than 10%, with a similarly modest decrease in resting ventilation.<sup>4</sup> Furthermore, when ventilation is unstimulated by exogenous carbon dioxide, conscious influences such as anxiety or merely thinking about one's breathing tend to perturb the measurements more than when ventilation is stimulated by an imposed increase in  $P_{aCO_2}$ . Finally, especially with rapidly acting drugs, it is not uncommon for apnea to occur; under these circumstances it becomes impossible to quantitate the ventilatory response until the carbon dioxide tension rises sufficiently to restimulate ventilation (fig. 1, *line B'*).

To overcome these obstacles, a variety of study designs have been used. These involve measuring the effects of medications on  $V_E$  measured at two or more imposed levels of hypercarbia. Such methods are termed *open loop* because the values of  $P_{aCO_2}$  are predefined and thus are unaffected by drug-induced changes in  $V_E$ . For long-acting drugs (or continuous infusions of shorter-acting drugs), both steady-state and rebreathing techniques can be used to determine the ventilatory response to artificially imposed hypercarbia. Rebreathing techniques involve simultaneous measurement of  $V_E$  and  $P_{aCO_2}$  while the subject's  $P_{CO_2}$  increases as a result of rebreathing from a closed system without carbon dioxide absorption. For steady-state measurements,  $V_E$  is measured after the subject equilibrates for 6–8 min at each of two or more elevated levels of  $P_{aCO_2}$  (typically between 46 and 58 mmHg). Romberg *et al.* used just such a steady-state technique in their comparison of the long-acting drugs morphine sulfate (MS) and M6G.<sup>2</sup> For single injections of short-acting drugs such as propofol or remifentanyl, isohypercapnic techniques have been described; ventilation is continuously measured during and after drug administration, whereas end-tidal pressure of carbon dioxide is held constant.<sup>5</sup> This method allows minute-to-minute determination of the carbon dioxide ventilatory response. If the pharmacokinetics of the drug are

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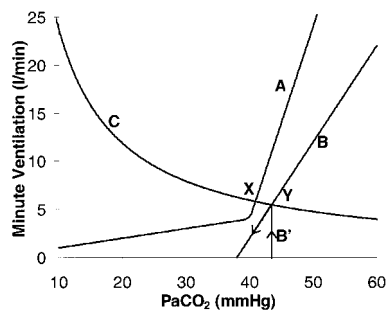


Fig. 1. Curve A represents the normal carbon dioxide response of an awake individual; the “hockey stick” appearance at low values of  $P_{aCO_2}$  corresponds to the observation that following hyperventilation, awake individuals do not become apneic but rather show a modest decrease in  $V_E$  until  $P_{aCO_2}$  returns to its resting value. Curve B represents the carbon dioxide response curve following administration of a sedative or anesthetic medication, which decreases its slope by 50%. Note that the curve no longer has a hockey stick shape but rather falls linearly to a  $V_E$  of 0 (the apneic threshold). Once apnea develops, the  $P_{aCO_2}$  must increase to approximately the resting value before ventilation restarts, accounting for the hysteresis loop (line B'). Curve C represents the carbon dioxide excretion hyperbola, which depends on the principle of conservation of mass: Assuming constant carbon dioxide production, increasing  $V_E$  will decrease  $P_{aCO_2}$ , whereas decreasing  $V_E$  tends to increase  $P_{aCO_2}$ . In the awake state, point X (the intersection of carbon dioxide response curve A with carbon dioxide excretion hyperbola C) defines the resting  $P_{aCO_2}$  and  $V_E$ , whereas point Y represents the values of  $P_{aCO_2}$  and  $V_E$  during sedation or anesthesia.

known, the effect compartment rate constant ( $k_{co}$ , which indicates how quickly the drug gets from the bloodstream to the site in the central nervous system where it causes respiratory depression) and potency ( $EC_{50}$ , which indicates the plasma concentration of the drug that causes a 50% decrease in ventilatory drive) of the drug can also be estimated. However, none of these techniques directly predicts what will happen to an actual patient, whose ventilation is not stimulated by exogenous carbon dioxide, when a rapidly acting ventilatory depressant is administered.

Bouillon *et al.* have overcome some of the shortcomings of previous closed-loop determinations of ventilatory drive by developing a mathematical model incorporating the pharmacodynamics of both carbon dioxide and remifentanyl.<sup>1</sup> Their model is designed to predict both the magnitude and time course of changes in  $V_E$  and  $P_{aCO_2}$  that would be expected to follow a remifentanyl-induced perturbation of the ventilatory response to carbon dioxide. To determine the pharmacodynamics of the carbon dioxide response, the investigators used a standard rebreathing technique. As indicated above, this method only provides information about that part of the carbon dioxide response curve which lies above the carbon dioxide excretion hyperbola (because ventilation is being stimulated by exogenous carbon dioxide). However, administration of a sedative drug in the absence of exogenous carbon dioxide will cause ventilation to drop below the carbon dioxide excretion hyperbola, as the carbon dioxide response curve acutely shifts down and

to the right. To estimate the relationship between  $V_E$  and  $P_{aCO_2}$  below the hyperbola, it was necessary to extrapolate. The authors demonstrated that their model fit the data best when the relationship between ventilation ( $V_E$ ) and  $P_{aCO_2}$  was expressed as a power function:

$$V_E = V_0 \left( \frac{P_{aCO_2}}{P_0} \right)^F$$

where  $V_0$  and  $P_0$  are the baseline values for  $P_{aCO_2}$  and  $V_E$ , respectively. The exponent F is an indicator of the “strength” of the carbon dioxide response before remifentanyl administration (analogous to the slope of curve A in the current fig. 1). As shown in Bouillon’s figure 2,<sup>1</sup> the carbon dioxide response lines predicted by this exponential model are curvilinear, resembling the “hockey stick” appearance of the awake carbon dioxide response.

To model the effect of remifentanyl on the carbon dioxide response, the authors assumed a sigmoidal relationship:

$$V_{Alv} = V_0 \left( 1 - \frac{C_p^\gamma}{C_{50}^\gamma + C_p^\gamma} \right)$$

which implies that at any given  $P_{aCO_2}$ , alveolar ventilation ( $V_{Alv}$ ) decreases from  $V_0$  (its baseline value in the absence of remifentanyl) to asymptotically approach 0 at high plasma concentrations ( $C_p$ ) of remifentanyl;  $C_{50}$  is the concentration at which ventilation is depressed to half its baseline value at any given  $P_{aCO_2}$ , whereas  $\gamma$  determines the “steepness” of the decline in ventilation with increasing  $C_p$ .

The second novel part of the model involves incorporating the pharmacodynamics and kinetics of carbon dioxide itself. Under the reasonable assumption that carbon dioxide production remains constant, the rate at which  $P_{aCO_2}$  increases as a function of  $V_E$  can be easily calculated; this is a generalization of the observation that during apnea  $P_{aCO_2}$  increases by 3–6 mmHg · min<sup>-1</sup> to situations in which ventilation is depressed but not zero. After incorporating an equilibration delay constant for carbon dioxide (to account for the ventilatory response to changes in  $P_{aCO_2}$  being not instantaneous), the authors closed the loop by substituting the sigmoidal prediction of  $V_E$  (their equation 11) into the equation predicting the rate of increase of  $P_{aCO_2}$  (their equation 9), to get their final model (their equation 13). With model in hand, the authors administered target-controlled infusions of remifentanyl, designed to achieve stepwise increases in remifentanyl plasma concentration. Based on frequent measurements of plasma remifentanyl concentration and  $P_{aCO_2}$ , they determined values for the parameters of their pharmacodynamic model that best fit the observed data.

The conclusions are fascinating and consistent with our observations in clinical practice. The plasma concen-

tration of remifentanyl predicted to cause a 50% decrease in alveolar ventilation at any given  $P_{aCO_2}$  ( $EC_{50}$ ) was  $0.92 \text{ ng} \cdot \text{ml}^{-1}$ ; this corresponds closely with previously published values of the concentration required to decrease ventilation by 50% during imposed hypercapnia.<sup>5,6</sup> More important, however, the authors demonstrate that the observed ventilatory effect depends on how quickly a given blood concentration is achieved. As shown in their figure 4, following a rapid  $0.5 \text{ } \mu\text{g}/\text{kg}$  dose of remifentanyl (peak plasma concentration  $\approx 5 \text{ ng} \cdot \text{ml}^{-1}$ ) the model predicts that ventilation will rapidly decrease to about 10% of its baseline value\*; within 10 min ventilation will recover and actually exceed its baseline value as the remifentanyl concentration drops in the presence of an elevated  $P_{aCO_2}$ . In contrast, if a similar blood level is gradually achieved by intravenous infusion ( $\approx 0.2 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 10 min), ventilation gradually decreases to a nadir of about 35% of its baseline value, before stabilizing at 60% of baseline. The reason for the discrepancy is that slow administration of the remifentanyl allows  $P_{aCO_2}$  to increase (to a predicted value in excess of 60 mmHg), partially offsetting the ventilatory depressant effect of the remifentanyl itself.

What are the clinical implications? For a given level of sedation, "bolus" administration of a sedative or opioid drug is more likely to cause more severe respiratory depression than gradual administration. During deep sedation or general anesthesia, administration of  $50 \text{ } \mu\text{g}$  of fentanyl to a typical adult almost always causes apnea, whereas administration of an equipotent dose ( $5 \text{ mg}$ ) of morphine typically causes modest respiratory slowing. The discrepancy can be explained by the slower onset of morphine, which allows  $P_{aCO_2}$  to gradually increase and stimulate ventilation. The distinction is important because gradual respiratory slowing minimally affects arterial oxygenation, whereas opioid-induced apnea is likely to produce hypoxemia unless the patient has been breathing an oxygen-enriched mixture.

Romberg *et al.* used more routine methodology to compare the ventilatory effects of MS and M6G; their thesis, based on ligand binding studies, was that M6G would produce less respiratory depression than an equianalgesic dose of MS. To prove their thesis, of course, it is first necessary to determine the relative analgesic potencies of MS and M6G. Previously published studies provide a wide range of potency ratios, depending on the species studied and route of administration. For example, whereas Person *et al.* found that  $0.05 \text{ mg}/\text{kg}$  of intravenous M6G was almost as effective as  $0.15 \text{ mg}$  of

intravenous MS in relieving experimental ischemic pain,<sup>7</sup> Lötsch *et al.* found that  $0.045 \text{ mg}/\text{kg}$  of intravenous M6G (followed by an infusion to maintain steady-state plasma concentrations) was ineffective in blunting the pain associated with nasal insufflation of 60%  $CO_2$ .<sup>8</sup> In the present study, Romberg chose a M6G dose of  $0.2 \text{ mg}/\text{kg}$ , based on unpublished data which suggested that it "caused potent and long-lasting analgesia," although no data are provided to establish equal analgesic efficacy with their  $0.13 \text{ mg}/\text{kg}$  dose of MS. Interestingly, owing to its higher molecular weight, the molar dose of M6G ( $0.43 \text{ } \mu\text{M}/\text{kg}$ ) was essentially the same as that of MS ( $0.46 \text{ } \mu\text{M}/\text{kg}$ ).

As shown in their figure 2, both M6G and MS significantly depressed the carbon dioxide ventilatory response; although there was no statistical difference between the time courses of the two drugs, it seems that the effect of M6G dissipated more rapidly than that of MS. Similarly, the peak effect on acute hypoxic response was similar between the two drugs, although the effect of M6G diminished more quickly than that of MS. Based on previously published data for the pharmacokinetics of MS and M6G, the authors then used pharmacokinetic/pharmacodynamic modeling techniques to estimate the  $EC_{25}$  (effect-site concentration causing 25% depression) and  $T_{1/2k_{co}}$  (the time required for the drug concentration within the central nervous system to reach half of its level in the plasma).

The authors' observation that the  $EC_{25}$  of M6G is 20–50 times greater than that of MS does not, by itself, imply that respiratory depression is less likely to occur with M6G than with MS. Rather, it is a consequence of differences in the pharmacokinetics of the two drugs. The volumes of distribution of M6G are appreciably smaller than those of MS. Thus, for any given dose, the plasma and effect site concentrations of M6G are necessarily higher than those of MS. However, these higher concentrations are also required to produce the desired, analgesic effect. Thus, Lötsch *et al.* found that plasma M6G concentrations of  $400 \text{ nM}/\text{l}$ , similar to those estimated by the present study to cause a 25% decrease in the ventilatory response to carbon dioxide, were completely ineffective in blunting experimental pain.<sup>8</sup>

Does the mechanism of ventilatory depression by M6G differ from that associated with MS? Romberg *et al.* found that for MS, the  $EC_{25}$  for the carbon dioxide response was greater than that for the hypoxic response ( $28.0 \text{ vs. } 16.5 \text{ nM}$ ), whereas for M6G, the reverse was true ( $528 \text{ vs. } 873 \text{ nM}$ ). However, neither confidence limits nor the results of statistical tests are provided, so it is impossible to determine whether this discrepancy implies that the two drugs act at different sites in the ventilatory control mechanism, or whether it is merely an example of type I error.

The other interesting finding of the Romberg study is that the effect-site equilibration of M6G ( $T_{1/2k_{co}} = 2.1 \text{ h}$ ) seemed to be more rapid than that of MS ( $T_{1/2k_{co}} =$

\* The model does not predict apnea, because the authors assumed a sigmoidal relationship between ventilatory depression and remifentanyl concentration; with such a model, the response is never completely abolished, even in the presence of very high drug concentrations. A power function model, such as that used by Romberg *et al.*, would be more likely to have predicted apnea, although it did not fit the observed carbon dioxide response data as well. Alternatively, separate modeling for the probability of apnea using probit or logistic analysis could be performed.



3.8 h). This seems to be inconsistent with M6G being a more polar molecule than MS and, hence, crossing the blood-brain barrier more slowly.<sup>9</sup> The authors suggest that this inconsistency may be related to differences in the transport of the two substances within the central nervous system. However, when pupil size rather than ventilation was used as a pharmacodynamic indicator, Lötsch *et al.* found that the  $T_{1/2k_{eo}}$  M6G was more than double that of MS.<sup>10</sup> A possible explanation for the discrepancy is that the volunteers developed tolerance to the respiratory depressant effects of M6G, artificially lowering the calculated  $T_{1/2k_{eo}}$  for M6G.

The key role of the blood-brain barrier in modulating the effects of M6G as compared with MS has been established by studies in which the substances have been injected directly into the subarachnoid space. In rodents, the analgesic potency of subarachnoid M6G is about 100 times greater than that of subarachnoid MS.<sup>3</sup> Grace and Fee found that the analgesic potency of subarachnoid M6G was about five times that of subarachnoid MS; however, respiratory depression was more likely in patients receiving M6G, again suggesting that use of M6G does not reduce the risk of respiratory depression.<sup>11</sup>

Thus, although Romberg *et al.*'s findings suggest that the respiratory depressant effects of M6G are similar to those of an equi-analgesic dose of MS, the data are not conclusive. Ideally, analgesic and respiratory depressant potency would be measured in the same subjects at a series of times after drug injection; pharmacodynamic modeling could then be used to determine if the respiratory margin of safety of M6G exceeds that of MS. Until such a comparison is undertaken, the utility of M6G as a respiration-sparing opioid analgesic remains a matter of speculation rather than reality.

Development of an opioid agonist devoid of ventilatory depressant effects remains the holy grail of analgesic pharmacology. Unfortunately, at least with regard to

M6G, the cup remains empty, because Romberg *et al.* were unable to demonstrate an increased margin of safety for this drug compared to nonselective  $\mu$ -opioid agonists such as morphine. However, Bouillon *et al.*'s study suggests that the risks of currently available nonselective opioids can be reduced through a more thorough understanding of the pharmacodynamics of ventilatory control. Their data demonstrate that by avoiding rapid changes in the concentration of depressant drugs in the respiratory control centers of the central nervous system, we can keep our patients *inspiring*, so they do not *expire*.

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## References

1. Bouillon T, Bruhn J, Radu-Radulescu L, Andresen C, Cohane C, Shafer SL: A model of the ventilatory depressant potency of remifentanyl in the non-steady state. *ANESTHESIOLOGY* 2003; 99:779-87
2. Romberg R, Olofson E, Sarton E, Teppema L, Dahan A: The pharmacodynamic effect of morphine-6-glucuronide *versus* morphine on hypoxic and hypercapnic breathing in healthy volunteers. *ANESTHESIOLOGY* 2003; 99:788-98
3. Lötsch J, Geisslinger G: Morphine-6-glucuronide: An analgesic of the future? *Clin Pharmacokinet* 2001; 40:485-99
4. Gross JB: Resting ventilation measurements may be misleading (Letter). *ANESTHESIOLOGY* 1984; 61:110
5. Babenco HD, Conard PF, Gross JB: The pharmacodynamic effect of a remifentanyl bolus on ventilatory control. *ANESTHESIOLOGY* 2000; 92:393-8
6. Glass PS, Iselin-Chaves IA, Goodman D, DeLong E, Hermann DJ: Determination of the potency of remifentanyl compared with alfentanil using ventilatory depression as the measure of opioid effect. *ANESTHESIOLOGY* 1999; 90:1556-63
7. Person RT, Simon PJ, Bakhshi K, Clark SJ, Langford RM, Slevin ML: Randomized placebo-controlled trial of the activity of the morphine glucuronides. *Clin Pharmacol Ther* 2000; 68:667-76
8. Lötsch J, Kobal G, Stockmann A, Brune K, Geisslinger G: Lack of analgesic activity of morphine-6-glucuronide after short-term intravenous administration in healthy volunteers. *ANESTHESIOLOGY* 1997; 87:1348-58
9. Bickel U, Schumacher OP, Kang YS, Voigt K: Poor permeability of morphine 3-glucuronide and morphine 6-glucuronide through the blood-brain barrier in the rat. *J Pharmacol Exp Ther* 1996; 278:107-13
10. Lötsch J, Skarke C, Schmidt H, Grösch S, Geisslinger G: The transfer half-life of morphine-6-glucuronide from plasma to effect site assessed by pupil size measurement in healthy volunteers. *ANESTHESIOLOGY* 2001; 95:1329-38
11. Grace D, Fee JPH: A comparison of intrathecal morphine-6-glucuronide and intrathecal morphine sulfate as analgesics for total hip replacement. *Anesth Analg* 1996; 83:1055-9

## A Glimpse Into the Many Possibilities that Lie Ahead

*Editor's Note:* In this issue of ANESTHESIOLOGY, Gamo *et al.* have used a series of very complex genetic and molecular genetic methods to identify and characterize, in *Drosophila melanogaster* (the fruit fly), a single gene that clearly plays a role in anesthetic sensitivity. Many of our readers might ask, "What do fruit flies have to do with 'real' anesthesia?" The answer is actually reasonably simple. Because of their rapid reproductive rate, and because of our extensive knowledge of *Drosophila* genetics, it is possible to conduct detailed exploratory and mechanistic genetic studies that would be difficult in any other species. In addition, fruit flies show complex behaviors that make it possible to define clear phenotypes. Finally, we now know that in many fundamental ways, fruit flies (and many "lower" organisms) are not as foreign from mammals and humans as we might initially expect. There is clearly a great deal that we can learn about the action of anesthetics on the nervous system by using such seemingly simple (but actually quite complex) models such as this.

I also quickly realized that despite the value of the science, perhaps 99% of our readers would find the methodology used by Gamo *et al.* to be completely unknown to them. For this reason, I asked Dr. Phil Morgan to write the following Editorial View. His assigned task was to explain to the educated anesthesiologist—who is nevertheless not an expert in modern genetics—exactly what Gamo *et al.* had done—and why they had done it. I think the result is a wonderful basic primer on some extraordinarily powerful modern tools, which we can only hope will be adopted by others as we continue our quest for a better understanding of the mechanisms of anesthetic action.

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CURRENT medical knowledge is increasingly based on the evolving fields of pharmacogenetics and pharmacogenomics. Genetic studies involving the mechanisms of anesthetic action and of the treatment of pain are of particular interest to anesthesiologists. Although it is evident that these involve complex mammalian physiology, it is becoming clear that homologous systems exist in simpler organisms. The importance of simple genetic models in establishing a core understanding of complicated mammalian systems cannot be overstated. In the present issue of ANESTHESIOLOGY, Gamo *et al.* have used such a genetic model, the fruit fly *Drosophila melanogaster*, to identify a gene that determines sensitivity to diethylether.<sup>1</sup> Our goal is to clarify the applicability of such studies to human medicine and to acquaint readers with the molecular genetic approach used by the authors.

### Applicability

Although many alternative approaches have made valuable contributions toward understanding how volatile anesthetics work, the use of molecular genetics in a whole animal model possesses two powerful and unique advantages. First, the DNA contained within virtually all cells dictates the structure of any anesthetic site, regardless of its chemical nature (*i.e.*, lipid, protein, or both). Second, by screening for mutations that alter responses

to anesthetics, nature directs the researcher to the important targets. As such, the data do not arise from preconceived ideas about what *should* be an anesthetic target.

The clinician may well wonder about the applicability to humans of the findings in a model organism. A conservative viewpoint acknowledges that because there will undoubtedly be some variation between organisms in the response to anesthetics, it is by comparing the results between these different systems that we are likely to gain more understanding of the global mechanisms by which volatile anesthetics function. However, the invertebrate model systems may actually provide more direct applicability than originally thought. Somewhat surprisingly, it seems that the human genome is much smaller and more similar to the genomes of nematodes and fruit flies than predicted.<sup>2</sup> A relatively high percentage of genes are conserved even across this wide variation in complexity of animals. Thus, in many ways, simple organisms often can be good initial models for molecular processes of more complex ones.

### Genetic Approaches

Classical forward genetics, as used by Gamo *et al.*, uses mutagens to cause heritable changes in the DNA of the experimental animal. The mutated animals are then screened for an observable change (phenotype) from normal, in this case, an alteration in anesthetic sensitivity. The genetic position of the DNA change is then "mapped" by mating animals containing the new mutation with other animals carrying mutations in known positions that confer visible phenotypes. By measuring the frequency of recombination between the mutations, a relative chromosomal position for the new mutation is obtained. *The beauty of this approach is its lack of preconceptions as to the molecular nature of a given trait.*

Molecular genetics, in turn, is used to analyze the

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nature of the mutation, relating it to the function of the normal gene product. Although still not a trivial undertaking, the task of dissecting the molecular mode of action of volatile anesthetics is substantially simplified by the use of genetic models. What should be the characteristics of such a tractable model? Of course, the organism must have observable behaviors that are disrupted by anesthetics. Preferably, these behaviors should be mediated by a nervous system functionally relevant to that of humans; however, a simple genetic system that can exploit the powerful tools of modern molecular genetic techniques is also needed. Ideally, the organism should have a well-mapped genome (lots of identified mutations already available) and a short generation time. These characteristics will allow for rapid gene mapping (as explained above). In addition, one would like to be able to create mutations in specific genes when desired. This technique, termed *directed* (or targeted) mutagenesis, allows for testing of particular genes when forward genetics has indicated that they might be important. In addition, a complete genetic sequence of the chromosomal DNA and a reasonable degree of homology of the genes from the organism to those of humans is also desirable. At present, the nematode *Caenorhabditis elegans* and the fruit fly *D. melanogaster* are the two organisms that generally satisfy the above requirements.

However, drawbacks also exist to such an approach. If a complicated pathway or cascade of events leads to particular behavior, one may have mutated any one of a great number of genes that may contribute to that behavior.<sup>3</sup> For example, mutations that change sensitivity to volatile anesthetics could arise from structural changes in molecules that *are* anesthetic targets, from changes in molecules that interact with an anesthetic target, or from a variety of changes that indirectly affect sensitivity. One must not, therefore, jump to the conclusion that every mutation that alters anesthetic sensitivity represents an anesthetic target.

The characterization of one such gene, *crc*, is presented in this issue of ANESTHESIOLOGY. The classical genetic mapping of *crc* was presented previously; the present work used molecular techniques to characterize *crc* as calreticulin—a multifunctional calcium binding protein. We will first describe the techniques used by the authors that allow isolation and identification of a gene along with characterization of its probable function. I will then describe the techniques that identify the expression of the gene, *i.e.*, when and where the gene product functions.

### Calreticulin

Most of the DNA in a genome is fixed in position, *i.e.*, the order of base pairs within genes does not change under normal conditions. In contrast, a P-element is a

transposon, or piece of DNA that can move around in the genome. The movement of transposons requires the presence of an enzyme, called a transposase, which is responsible for the transposons jumping in and out of genes. By inserting itself into a gene, a transposon is capable of disrupting the order of the base pairs and causing a mutation in that gene. The authors used P-elements to cause random mutations in the fly genome and characterized one that altered the anesthetic sensitivity of the fly.

Modern day P-elements have been engineered to include some bells and whistles that provide the investigator with a “tag” to identify the location of the P-element insertion. The authors isolated the gene by using the fact that the P-element also contained an antibiotic resistance gene (like the antibiotic resistance plasmids we hear so much about). The P-element and accompanying DNA (from the gene) was cut out of the fly genome, and the resulting fragment (now a plasmid) was placed in bacteria that were grown in the presence of the antibiotic. Only the bacteria containing that plasmid survived exposure to the antibiotic (plasmid rescue); the same plasmids contained the accompanying DNA from the fly gene. As the rescued bacteria multiplied, they generated multiple copies of the plasmid DNA (with the accompanying “cloned” gene sequence).

By adding a transposase (the enzyme that mobilizes transposons) back into the mutant background, the authors caused the P-element to jump out of the *crc* gene. This generated normal animals from the mutant *crc* parents (termed *revertants*; that is, animals whose phenotype had reverted to normal). This technique proved that it was the insertion of the P-element into *crc* that caused the altered anesthetic phenotype. In addition, once the gene fragment accompanying the P-element was obtained, that fragment was used to identify the entire gene, information available because the entire *Drosophila* genome has been sequenced. The normal gene was then reintroduced into a mutant fly to create a “rescued” transgenic animal that now had a normal anesthetic phenotype. Mutant rescue is usually taken as proof that the gene being studied is the one altered to cause the mutant phenotype.

To convert DNA sequence into protein, the DNA is first transcribed into an intermediate *pre-messenger* RNA (pre-mRNA) molecule. This undergoes further processing *in vivo* wherein noncoding regions of the gene are eliminated from the sequence (splicing) to generate the final mRNA. Unlike the mRNA whose sequence is complementary to that of the original DNA strand, a cDNA is an exact copy of the DNA strand transcribed from the gene. Like mRNA, it only contains the protein coding sequences from the gene. It is synthesized *in vitro* using the mRNA as a template. The authors screened a collection of clones of all such cDNAs from the fly using the partial fragment from the *crc* gene (obtained earlier *via*

plasmid rescue) to isolate the cDNA corresponding to the *crc* gene. Later, they verified that the isolated clone was complete by *in vitro* synthesis of the two ends of the *crc* cDNA using a technique called RACE (rapid amplification of cDNA ends).

Once the sequence of base pairs in the gene was obtained (by automated DNA sequencing) and compared across species, the function of the protein product became clear. Genes coding for similar proteins in different organisms often have a similar order of bases, and the authors found that the base sequence of the mutated gene was very similar, or homologous, to the gene calreticulin studied in other organisms. The degree of homology left little doubt as to the identity of this gene (calreticulin/*crc*) in *Drosophila*.

The above techniques identified the position, sequence, and probable function of the *crc* gene (*i.e.*, calreticulin) and proved that *crc* was responsible for the mutant phenotype. The authors then continued to further characterize *crc* by determining when and where the gene was used during development. mRNA can be size-separated on agarose and transferred and immobilized onto a membrane, a technique called the Northern blot. By probing mRNA isolated from animals synchronized at various stages in development (developmental Northern blots) with a specific gene fragment, one can obtain information regarding temporal expression (the “when”) of that particular gene. By using a *crc* specific probe on developmental Northern blots, the authors showed that *crc* mRNA was made (expressed) at variable levels for most of the life of the fly.

Inherent within the DNA sequences of most genes are regulatory elements called promoters that control the temporal and spatial expression of that gene. Some of these are active only under selected conditions. An example is the promoter from heat shock protein that is strongly active only at elevated temperatures (37°C). By placing *crc* under the control of a heat shock promoter, the authors obtained conditional expression of the *crc* gene product in the transgenic strains by raising or lowering the temperature at specific times during development. Their results were consistent with the gene being necessary throughout the life of the fly. However, because the heat shock promoter is known to be somewhat leaky (*i.e.*, also functions weakly at lower temperatures), some expression occurs even in the absence of heat shock. As a result, low levels of expression cannot be ruled out as sufficient for survival.

*In situ* literally means “in the original position.” Hybridization of tagged nucleic acid probes to RNA *in situ*

is a powerful method to analyze the “where” of gene expression in embryos or tissues. Embryos collected at regular intervals (or tissues) are fixed using techniques that preserve their cellular morphology. Subcellular distribution of a particular mRNA can then be studied using a tagged nucleic acid probe specific to that gene with a sense probe (that represents the nontranscribed DNA strand from that gene) serving as an effective control. By comparing gene expression patterns in normal and mutant animals, one can discern a role for the particular gene product during development. The authors used this technique to analyze *crc* expression in fly embryos and tissues. Consistent with the results of the developmental Northern blots, they found that *crc* was expressed at varying levels throughout the life of the fly. In addition, they found that the expression was ubiquitous in the embryo but was largely restricted to the nervous system in the larva and in the adult fly.

In conclusion, Gamo *et al.* have used a simple model system to identify a gene, known to function in mammals, as important in determining anesthetic response. Furthermore, they have shown that this gene functions in the nervous system and is important for the entire life of the fly. This work shows that the deluge of new tools to analyze gene expression and function continues to improve the ability of investigators to distinguish the finer details of gene action. The disparate, albeit discrete, set of previously unconsidered anesthetic targets, identified by such molecular genetic studies in simple organisms, attests to the remarkable power of such an approach in unraveling complex mechanisms. Although interspecies comparison of anesthetic targets has not yet been possible, these candidates offer us a glimpse into the many possibilities that lie ahead. The use of such models in dissecting other complex behavioral problems, such as perception of and response to pain, offers a tantalizing prospect for future endeavors.<sup>4</sup>

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## References

1. Gamo S, Tomida J, Dodo K, Keyakidani D, Matakatsu H, Yamamoto D, Tanaka Y: Calreticulin mediates anesthetic sensitivity in *Drosophila melanogaster*. *ANESTHESIOLOGY* 2003; 99:867-75
2. Ewing B, Green: Analysis of expressed sequence tags indicates 35,000 human genes. *Nat Genet* 2000; 25:232-4
3. Campbell DB, Nash HA: Use of *Drosophila* mutants to distinguish among volatile general anesthetics. *Proc Natl Acad Sci USA* 1994; 91:2135-9
4. Kellenberger S, Schild L: Epithelial sodium channel/degenerin family of ion channels: A variety of functions for a shared structure. *Physiol Rev* 2002; 82: 735-67



## Is it Time to Get on the Fast Track or Stay on the Slow Track?

MYLES *et al.* performed a meta-analysis of all randomized trials of adult cardiac surgery in which patients underwent coronary artery bypass graft surgery with cardiopulmonary bypass and/or vascular surgery with cardiopulmonary bypass to examine the question of whether anesthetic technique affected the outcome of cardiac surgery. Ten randomized trials met the inclusion and exclusion criteria for adequacy of study design with a total of 1,800 patients. It is a bit surprising that only 10 randomized trials of anesthetic technique could be found in the world literature for a surgical procedure that is performed more than 500,000 times a year. The even more surprising finding is that 1,012 of the 1,800 patients came from a single study. More than 56% of the total patients studied in randomized trials in the world literature for anesthetic technique in cardiac surgery came from a single study by Slogoff and Keats published in 1989.<sup>1</sup> Does this affect the results of Myles *et al.*?

Slogoff and Keats<sup>1</sup> performed a prospective, randomized, blinded-analysis, clinical trial of four different anesthetics—sufentanil (15–30  $\mu\text{g}/\text{kg}$ ) or fentanyl (10  $\mu\text{g}/\text{kg}$ ) in combination with isoflurane, halothane, or enflurane—and found no difference in primary outcome myocardial infarction or death. For a study that was underpowered to demonstrate a difference in hard outcomes, it is not surprising that none was found. The authors calculated that 7,844 patients would need to be studied to have an 80% power to show a difference in myocardial infarctions and 3,287 for death. It is not surprising, given the calculated sample sizes, that no further large-scale trials of anesthetic agents were designed with myocardial infarction or death as an endpoint.

Slogoff and Keats also used surrogate endpoints. They found no difference in their surrogate outcome variables of myocardial ischemia or creatine phosphokinase and isoenzyme release of creatine kinase containing M and B subunits between the anesthetic techniques. They found that tachycardia led to ischemia, ischemia led to ischemia, and  $\beta$ -blockers reduced tachycardia. They also found that sufentanil 15–30  $\mu\text{g}/\text{kg}$  caused longer periods of tracheal intubation ( $22.8 \pm 12.3$  vs.  $15.3 \pm 6.3$ ,  $P = 0.001$ ) than fentanyl (10  $\mu\text{g}/\text{kg}$ ) in combinations with an inhaled agent. They explained that the “. . . duration[s] of

postoperative intubation were as expected from the known pharmacologic effects of the primary anesthetic agents.” The definitive work on the use of fast-track *versus* slow-track cardiac anesthesia was published in 1989, with the patients studied between 1985 and 1987.

What additional information can we glean from the subsequent 15 yr of experience with fast-track anesthesia? Myles *et al.* conclude that fast-track anesthesia is safe. No significant outcome differences were found in 30-day all-cause mortality, myocardial infarction, sepsis, wound infection, stroke, acute renal failure, prolonged intensive care unit stay, or surgical reexploration for bleeding. The duration of tracheal intubation and intensive care unit length of stay were shorter for fast-track cardiac anesthesia. As expected, the duration of postoperative intubation was shorter, given the known pharmacologic effects of the primary anesthetic agents.

Unless someone decides to study between 3,000 and 8,000 patients, the world literature will be left with the conclusions of Slogoff and Keats that there is no definitive difference between high-dose opioid anesthesia and low-dose opioid anesthesia with an inhaled agent. That is not to say there is no difference—there is just little chance of showing a difference without studying very large numbers of patients. It is important to remember that the failure to demonstrate a difference is not proof of similarity. The two techniques could still be different, but the difference must be small. On the other hand, demonstrating a statistically significant difference indicates a high probability of a true difference. When deciding one of the fundamental questions of anesthetic technique, we are left with the conclusions of Slogoff and Keats. Controlling the heart rate prevents ischemia, preventing ischemia prevents myocardial infarctions and deaths, and the best way to prevent myocardial ischemia is with a  $\beta$ -blocker, not with the choice or dose of opioids. There is no benefit from high-dose opioid anesthesia for cardiac surgery as compared to low-dose opioid anesthesia with an inhaled agent. The low-dose opioid anesthesia with an inhaled agent allows for fast-track cardiac surgery, which may reduce costs. The surrogate endpoints of a 15-yr-old study are as important today as they were in the past. Myles *et al.* have provided an important systematic review of the world literature on the safety and effectiveness of fast-track *versus* slow-track cardiac anesthesia. It is time to get on the fast track.

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### Reference

1. Slogoff S, Keats AS: Randomized trial of primary anesthetic agents on outcome of coronary artery bypass operations. ANESTHESIOLOGY 1989; 70:179–88



## *On Behalf of the American Society of Anesthesiologists Committee on Practice Parameters*

IN 1989, the Agency for Healthcare Policy and Research (AHCPR) was created by public law. In 1990, Richard Stein, M.D., President of the American Society of Anesthesiologists (ASA), was advised by an agency within the AHCPR of new legislation to develop, review, and update clinical practice guidelines. In 1991, the ASA established an Ad Hoc Committee on Practice Parameters.

Practice standards are authoritative statements or rules of minimum requirements for clinical practice. Standards for Basic Intraoperative Monitoring are the classic example (e.g., pulse oximetry). A standard may be modified only under unusual circumstances (e.g., extreme emergencies).

Practice guidelines are systematically developed recommendations for patient care that describe a basic management strategy or a range of basic management strategies. Guideline recommendations are supported by analysis of the current literature and by a synthesis of expert opinion, open forum commentary, clinical feasibility data, and consensus surveys. Guidelines are not intended as standards or absolute requirements. They may be adopted, modified, or rejected according to clinical needs and constraints.

Practice advisories are systemically developed reports that are intended to assist decision making in areas of patient care in which scientific evidence is insufficient. Advisories provide a synthesis and analysis of expert opinion, clinical feasibility data, open forum commentary, and consensus surveys. Advisories are not intended as standards or guidelines. They may be adopted, modified, or rejected according to clinical needs and constraints.

Before 1990, the ASA had produced Standards and Guidelines. The best known of these was the Standard for Basic Anesthetic Monitoring, which was first approved in 1986. Guidelines had also been developed for Ambulatory Care, Continuing Medical Education, Critical Care, and others. However, the new parameters were to be designed to address clinical practice pathways relating "how to, when to," and so forth. In many ways, this was medicine's response to the utilization by insurance

and managed care companies of "black box" methodology to retrospectively deny or allow charges for care.

The Ad Hoc Committee was composed of anesthesiologists selected by type of practice and geographic location. The Committee would select a topic for a parameter, obtain approval from the Board of Directors and the House of Delegates (HOD), and then recommend a task force to be appointed by the president of the ASA. A chairman would be appointed, and a methodologist trained in developing parameters would be hired as a consultant. A comprehensive literature search would be done and the guideline would be developed based on evidence-based data, in addition to consensus or expert opinion. A draft would be developed and circulated to experts and other interested anesthesiologists. An open forum or two would be conducted at a major anesthesia meeting to obtain feedback. The completed document would then be presented to the HOD of the ASA, where it could be approved or rejected but not modified on the floor of the House. This has been one of the most important reasons for any success this venture has had.

After 2 yr of experience in hiring consultants as methodologists, our committee recommended that a full-time methodologist be hired as a cost-saving measure. Richard Connis, methodologist for the Difficult Airway Guidelines from 1991-1993 was hired first, and in 1994 David Nickinovich was also employed. These two Ph.D. methodologists work in Seattle, Washington, under the direction of Robert Caplan, M.D.

To obtain wide dissemination of our parameters, we have been fortunate to have each one accepted for publication by ANESTHESIOLOGY. When the ASA approved the concept of practice parameters in 1990, it was suggested that the parameter should be revised or subject to sunset laws after 5 yr. The Agency for Healthcare, Research and Quality (the renamed AHCPR) established a National Guideline Clearing House for parameters, which has accepted each of the ASA's products. The public seems to use this Clearing House as the first source to access most parameters. However, the Clearing House omits any parameter that has not been updated in a 5-year time frame. Obviously, timely revisions are important.

Our committee has intentionally allowed the time frame to increase for two reasons. First, if the 5-year time frame were adhered to, it would be necessary to initiate the revision only 3 yr after publication. Second, a significant outlay of money is required to revise a parameter, and our members volunteer many hours of their valuable time. What about the Clearing House? Most, if not all of

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our guidelines, are directed to anesthesiologists and not the public. However, our guidelines do receive a number of hits each month, which I expect are from the public or other healthcare professionals who do not routinely access ANESTHESIOLOGY. We have made the decision to increase the time frame and are willing to accept any potential downside.

The issue of legal consequences always comes up. The general impression is that more cases have been won than lost because of guidelines. In anesthesia, the major problem has been when monitoring or equipment is way below standard and a bad result occurs after treatment. Proper documentation is key in avoiding adverse legal action. No member of our committee has ever been asked to testify on guidelines in an official capacity; if one were to testify, that member would testify as an individual. Certainly, the member's position on the committee would undeniably add weight to the testimony.

A task force was convened to develop a guideline on the "Ideal Practice of ANESTHESIOLOGY." However, after considerable effort and discussion, the task force elected not to develop such a guideline, which they believed already existed in previous statements issued by the ASA. The ASA HOD has also rejected a guideline. However, to meet the concerns of our members, the task force rewrote the guideline, which passed in the next year. As chair of the Committee, my view is that the HOD gives final approval to the topics selected. A best faith effort is used to develop a good guideline. The HOD has the ultimate authority and responsibility to approve or reject the guideline.

It is very difficult to develop a guideline authored by multiple societies. The problems mount in geometric proportions. In obstetrics, a turf war developed over the

words *immediately available*. The strategy has been to select a task force and appoint members of other specialties as consultants to our task force as individuals and not as official representatives. With the exception of the Conscious Sedation Guideline, ASA has not sought the endorsement of other specialty societies.

The committee will seek HOD approval in October to develop a guideline on sleep apnea. Approval is also being sought to develop a guideline on pacemakers and implantable defibrillators. Consideration has been given to developing a guideline on pediatric anesthesia. However, it has been decided not to proceed to avoid the conflict about "who should administer anesthesia to a pediatric patient." If a member believes a specific guideline should be developed, that member may contact me as chair, or any member of the committee. The ASA Newsletter includes an annual update on practice parameters to communicate to members the plans for either new guidelines or guideline revisions.

In summary, the guideline movement will continue as long as the HOD approves both the activities and the funding. Every effort is being made to be fiscally prudent. Fewer meetings are being held for each guideline being developed, but some face-to-face meetings must be held to clarify controversial issues. It takes approximately \$150,000 to develop each guideline. This is substantially less than what other organizations spend per guideline, mainly because of the willingness of our members to volunteer their valuable time in developing a guideline. I hope this will provide our readers with more insight into the guideline process.

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