Mapping Anesthesia Genes

Why and How?

TO define general anesthetic mechanisms, one must be able to study general anesthesia. This somewhat inane statement nevertheless points out a fundamental problem in anesthetic mechanism research: the difficulty in relating molecular level anesthetic effects to a behavioral endpoint approximating anesthesia. Genetics is a powerful tool for bridging this molecular to behavioral gap. Recent successful inroads have been made using the fruitfly Drosophila melanogaster and the nematode Caenorhabditis elegans. Both models have shown that anesthetic sensitivity can be genetically manipulated and that the differences in sensitivity can be mapped to chromosomal regions.\(^1\)\(^\text{--}\)\(^8\) Given their molecular genetic power and their well-defined simple nervous systems, the invertebrate models offer the best tools for describing the full depth and breadth of anesthetic mechanisms. However, genetic models more closely related to humans are clearly needed to confirm the findings from invertebrates and to identify any vertebrate-specific mechanistic components. In this issue of Anesthesiology, Simpson et al.\(^7\) report the first mapping in a vertebrate species of a gene or genes that affect anesthetic sensitivity. Given that genetics is not the forte of most anesthesiologists, this editorial aims to make the genetic methods used by Simpson et al. more understandable to the non-expert.

Genetic approaches can be divided into three basic types. Classical genetics is that with which we are all to some degree familiar. Here, a mutation in a single gene results in an individual that is qualitatively different from the non-mutant (wild type) individual. A second approach, reverse genetics, is becoming an increasingly powerful and used method because of the number of sequenced genes without a well-defined function. A known gene is directly mutated, and then the functional consequence of that mutation (i.e., the phenotype) is determined. Thus, the process goes from gene to phenotype in reverse from classical genetics. Transgenic “knockout” mice is a reverse genetic technique. For example, the 6 subunit of the GABA\(_\alpha\) receptor has been functionally disrupted or “knocked-out.” The 6-deficient mice had normal sensitivities to ethanol, pentobarbital, enflurane, and halothane, suggesting that the 6 subunit is not required for sensitivity to these anesthetics.\(^8\)

The third approach, that used by Simpson et al., is quantitative genetics, which is the study of quantitative or continuously varying traits such as height and weight.\(^9\) Usually, quantitative traits are controlled by multiple genes, each called a quantitative trait locus (QTL) because each contributes quantitatively to the net trait. With its ability to examine multiple genes simultaneously, quantitative genetics may be particularly useful for the study of anesthetic mechanisms because of the many potential anesthetic targets\(^10\) perhaps acting in concert to produce anesthesia. Natural genetic variation (i.e., not induced by mutagens) in evolutionary divergent strains provides the sequence differences in multiple genes needed for quantitative genetic studies.

The genetically variant strains used in the Simpson study were mice selectively bred for their difference in sleep time after intraperitoneal injection of ethanol.\(^11\) Subsequently, these long-sleep (LS) and short-sleep (SS) mice were also found to be hypersensitive and resistant, respectively, to propofol.\(^12\) SS mice not only regained the ability to remain upright faster after propofol injection but also “woke up” at higher brain levels of propofol;\(^12\) thus, differential nervous system sensitivity rather than metabolism was responsible for the difference in propofol sleep times.

In their current article,\(^7\) for the purposes of genetically mapping the variation in propofol sensitivity, the SS and LS strains were mated to produce F2 and recombinant inbred strains (RIs). Figure 1 shows how one makes F2s and RIs and how they can be used to map a locus. In this hypothetical example, two highly inbred
Fig. 1. Mapping genetic loci in F2s and RI strains. A hypothetical set of inbred strains differs for the GAS locus. Strain A carries the resistance allele, strain B the sensitive allele, and they also differ for two marker sequences M1 and M2 that can be detected by PCR. Mating of A with B produces first generation F1 individuals that are genetically identical. During meiosis, the F1 chromosomes recombine (X) at random locations, sometimes more than once. Mating of F1s produces F2s with various combinations of randomly recombined chromosomes. Most chromosomes have no recombination between the M1 marker and the GAS locus. Thus, most MIA homozygotes are anesthetic resistant; most MIB/M1B animals are sensitive. Note the exception at the right where a recombination has occurred between M1 and GAS. Mating of brother-sister pairs for 20 more generations produces RI strains that are homozygous at over 97% of their genome because of pairing of identical chromosomes in 1/4 of the progeny in each generation. Thus, in this example where only one locus is controlling anesthetic sensitivity, each RI strain is either resistant or sensitive and homozygous for either the A or B version of the markers. For illustrative purposes, recombination (X) is shown to occur only in F1s. In actuality, recombination continues at every generation; thus, the RI strains have more recombined chromosomes than F2.

animal strains differ in their anesthetic sensitivity. This difference is a result of a single gene, called here the GAS locus. Strain A carries the anesthetic resistance conferring version or allele of the GAS locus, and strain B carries the anesthetic sensitive allele. The single chromosome of this hypothetical animal can be distinguished in the two strains at two different locations, M1 and M2. These “markers” are typically previously mapped short DNA segments that differ in size in the two strains (e.g., 200 base pairs in A and 500 base pairs in B). By using polymerase chain reaction (PCR), this difference in size can be detected by amplifying the segment with flanking DNA primers and determining the size of the amplified DNA on a polyacrylamide gel. Strains A and B can be mated to one another to produce F1 animals. During F1 gametogenesis, recombination occurs between the homologous chromosomes at random and sometimes multiple locations. These recombinant chromosomes produce unique F2 individuals with a mixture of chromosomal segments and, therefore, genes from strain A and strain B. If these individuals are tested for their general anesthetic sensitivity, some are resistant (two R alleles), some are sensitive (two S alleles), and some are intermediate (one R and one S allele, assuming no dominance of one allele over the other). A correlation of markers with phenotype would show that the MIA marker version correlates with anesthetic resistance and the M1B with sensitivity. A much weaker correlation would be seen with the M2 marker types because the M2 marker is genetically farther away from the GAS locus. Hence, an increased number of recombination events will occur between the marker and the locus. The GAS locus would be said to be linked to the M1 marker. The statistical tests used to determine linkage are many, but the simplest conceptually is analysis of variance (ANOVA). In this case, the values of the measurements of anesthetic sensitivity (e.g., duration of loss of righting reflex) in the strains with the M1A marker are compared with those with the M1B marker. A statistically significant difference between the two groups demonstrates linkage. Simpson et al. used a statistical method called interval mapping to demonstrate linkage. Unlike ANOVA, interval mapping considers two adjacent markers simultaneously and estimates the QTL location more precisely by interpolation between the markers.

As shown in figure 1, F2s can be further bred to give what are called recombinant inbred strains (RI). RI strains are produced by brother-sister mating at each successive generation so that eventually the majority of the genome is homozygous (>97% homozygous after greater than 20 generations of inbreeding). The main advantage of RIs over F2s for gene mapping results from their genetic stability as homozygotes. Thus, subsequent generations can be repeatedly tested for the phenotype of interest without a change in their genetic composition. They also can be later tested for other phenotypes, and as they become available, additional markers can be scored to improve the precision of mapping. Also, although not indicated in figure 1, each generation will accumulate additional recombination events until homozygous as RIs. More recombinations between the markers and the locus increase the power and precision of mapping. Otherwise, the basic principles of mapping loci in RI by recombination frequency with markers are the same as those for F2s. Simpson mapped the propofol locus (called Lorp1 — Loss of Righting due to Propofol) in F2s and RIs. Mapping the variation in propofol sensitivity in the RI strains produced a much more statistically significant and precisely mapped QTL than in the F2s. However, both methods pointed to the same region on mouse chromosome 7 near the albino locus. All pigmented RIs (the LS strain is pigmented, the SS strain is albino) were relatively sensitive to propofol when compared with albino RIs. Thus, Lorp1 is tightly linked to, although not necessarily
the same as, the tyrosinase gene, which when mutated produces albinism.

Demonstration that propofol sensitivity is heritable and can be mapped to a genetic locus is by itself significant, but the real payoff will come with identification of the gene or genes that form Lorp1. Some of the genes, like the tyrosinase gene, within the relatively tight 99% confidence interval for the Lorp1 locus have been identified. Additional candidate genes will be found with the systematic sequencing of the mouse genome. Reasonable candidate genes can be examined for sequence differences in the SS and LS strains. Through this process, prospects for identification of the Lorp1 locus seem favorable. We may then make a causal link between general anesthesia and the molecules active in producing it.

C. Michael Crowder, M.D., Ph.D.
Departments of Anesthesiology and Molecular Biology/Pharmacology
Washington University School of Medicine
St. Louis, Missouri
crowderm@morpheus.wustl.edu

References