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TITLE: **ISOFLURANE RESISTANT MUTANTS OF *SACCHAROMYCES CEREVISIAE***

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The mechanism by which volatile halogenated anesthetics act at the cellular level is poorly understood and the site of action for these drugs remains undefined. Use of a molecular genetic approach to this problem could provide information about the pathway(s) involved and the site(s) of anesthetic action. A mutant organism with an altered sensitivity to halogenated anesthetics must contain DNA that codes for an altered gene product. Identification of this gene or gene product would allow construction of an hypothesis about how anesthetics interact with the cell. The yeast, *Saccharomyces cerevisiae*, a small eukaryote, shares many similarities with mammalian cells and is widely used to study processes of eukaryotic cells. Because of its ease of handling, its suitability for using molecular technology and its well characterized genetic system, we have chosen *S. cerevisiae* as a model to study the mechanism of anesthetic action. Growth of wild-type *S. cerevisiae* is inhibited by the inhaled anesthetic isoflurane, although the cells are not killed by this compound. We have isolated mutations in two different genes, *ISO1* and *ISO2*, that permit yeast to grow in the presence of isoflurane.

Yeast cells plated on solid media were exposed to 14% isoflurane for 4 days in a gas tight chamber. This high concentration was used to completely inhibit growth and allow mutants resistant to isoflurane to be easily selected. Concentrations of isoflurane were confirmed at 12 hour intervals using Raman spectroscopy. Three mutants (**M1**, **M2**, and **M3**) were isolated that grow in the presence of isoflurane while growth of the wild-type strain is arrested until isoflurane is removed from its environment.

Crossing the mutants to wild-type strains of the opposite mating-type and dissection of the products of meiosis revealed 2 resistant and 2 sensitive progeny confirming that all three mutants result from mutations in single nuclear genes. Testing the diploid cells revealed all to be isoflurane sensitive demonstrating that the mutations are recessive. Two of the mutants, **M2** and **M3** did not grow well at 38°C, while the wild-type and **M1** grew normally. Temperature sensitivity and isoflurane resistance cosegregated indicating both phenotypes are caused by the same mutation. Temperature-sensitive mutants normally identify genes which are essential to the cell. Analysis of crosses between the mutants show that **M1** and **M3** are different alleles of the same gene (*ISO1*), and **M2** defines a different gene (*ISO2*).

By cloning and sequencing the *ISO1* and *ISO2* genes, homologies to other characterized genes can be determined using sequence analysis. Any homologies may provide insight into the mechanism of action of isoflurane. The cloned genes can also be used to search for similar sequences in higher eukaryotic organisms.

Summary of Mutant Characteristics

Strain	cfu[1] at 14% Isoflurane	Growth at 24°C	30°C	38°C	Allele Designation
M1	100%	++	++	++	*iso1-1*
M2	1%	++	++	+-	*iso2-1*
M3	10%	++	++	--	*iso1-2*
wild-type	<0.01%	++	++	++	

All of the mutations are inherited as recessive alleles of single nuclear genes.

[1]colony forming units (cfu) are determined by plating appropriate dilutions of cells in the presence and absence of isoflurane and determining the % of colonies growing after 4 days of exposure to isoflurane.

-- = no growth; +- = very poor growth; ++ = wild-type growth

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Title: WHAT ROLE DO ALPHA ADRENERGIC SYSTEMS PLAY IN TONIC MODULATION OF SPINAL SENSORY PROCESSING?

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Research is leading to a better understanding of the complex pharmacology of spinal sensory processing. Opiate, alpha adrenergic and serotonergic receptors are now known to have a profound influence on the processing of afferent information at the level of the spinal dorsal horn. We have recently[1] reported that pharmacologic blockade of serotonergic systems has a profound influence on the response of some neurons to stimulation of their peripheral receptive fields. Those results indicate that, in cat spinal cord, serotonin is important for tonic control of afferent information. At the present time, there is great interest in the use of alpha adrenergic agonists in anesthesia. The purpose of this ongoing study is to better define the effect of alpha adrenergic systems on spinal sensory processing.

This protocol was approved by the Yale Animal Care and Use Committee. Extracellular recordings of single spinal dorsal horn neurons were made in physiologically intact, awake, drug-free cats before and after drug administration (each cell served as its own control). Following baseline studies of neuronal response, the alpha-2 adrenergic antagonist idazoxan was administered intravenously (0.075-0.3 mg/kg). The receptive field area that was sensitive to light touch was mapped before and after drug administration. Neuronal responses to brushing, pinching and heating of the receptive fields were also determined before and after I.V. drug administration. In five neurons, propofol (7.5 mg/kg) was administered intravenously after the effect of idazoxan had been studied and neuronal responses to the same stimuli described above were examined to compare the effect of propofol with that produced by idazoxan.

To date, ten low threshold neurons have been studied. Intravenous idazoxan produced no obvious changes in receptive field size or responses to brushing, pinching and heating. Propofol decreased spontaneous firing rates, receptive field size, and response to brushing or non-noxious pinching 73%, 52%, 53%, and 25%, respectively. These alterations resulting from propofol administration are consistent with our previous report.[2] Idazoxan did cause an increase in background neuronal activity of 9 of the 10 neurons studied. Although the percent increase in spontaneous activity was large, because the baseline values were very low, the physiologic significance of the small real change is questionable.

Alpha adrenergic agonists have been shown to have a profound effect on neuronal activity within the spinal dorsal horn. The results of this ongoing study suggest that alpha adrenergic modulation of spinal sensory processing in intact cats may not be tonically active. Unlike the serotonergic system, the administration of a selective alpha adrenergic antagonist caused no significant change in neuronal response to peripheral receptive field stimulation. The absence of idazoxan effects on neurons that were inhibited by propofol further suggests a lack of tonic alpha adrenergic effect at the spinal level. It is possible that the alpha adrenergic system is specialized to modulate spinal sensory processing only under specific conditions. It may function as a special system activated by stress or pain to depress pain signals at the level of the spinal cord rather than being tonically active.

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References:
1. Pain 40, 205-19, 1990
2. Anesthesiology 73, A697, 1990