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Rapid communication

The site of general anesthesia and cytochrome *P*450 monooxygenases: occupation of the enzyme heme pocket by xenon and nitrous oxide

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Abstract

In previous studies, cytochrome *P*450 monooxygenases were shown to be appropriately sensitive to structurally diverse compounds varying widely in anesthetic potencies and to increasing carbon-number series of straight chain primary and secondary alcohols and rigid cyclic alcohols. We now report that xenon and nitrous oxide, at one atmosphere, occupy the *P*450 heme cavity and competitively inhibit catalytic activity. The heme enzymes appear to be the most relevant model of the site of general anesthesia, thus far identified.

Keywords: General anesthesia; Cytochrome *P*450; Xenon

A crucial milestone on the path to revealing the putative, common molecular target that mediates the action of general anesthetics would appear to be identification of a biomolecule with appropriate sensitivity to the entire array of anesthetic compounds. We showed occupation of the heme cavity and inhibition of catalytic activity of cytochrome *P*450 monooxygenases by relevant concentrations of an extensive, diverse collection of compounds, that vary in anesthetic potencies by several orders of magnitude (LaBella et al., 1998). Furthermore, we found that *P*450 enzymes exhibit a remarkable correspondence with the site of general anesthesia with respect to (a) an absolute sensitivity to increasing chain-length series of flexible, straight chain primary and secondary alcohols and straight chain diols, (b) an absolute sensitivity to increasing molecular weight series of rigid cyclic alkanols and cyclic alkanemethanols, (c) the points of abrupt change and of reversal (cut-off) in the linear relationship between increasing anesthetic potency with increasing carbon chain length, and (d) non-differentiation between secondary alkanol enantiomers (LaBella et al., 1997). If one accepts the criterion that the site of general anesthesia and its biomolecular counterpart must share the same sensitivity to all chemical agents that effect anesthesia, then cytochrome *P*450 monooxygenases represent the most relevant model identified, thus far.

The GABA receptor is a macromolecular complex that represents a putative mediator for many, but not all, general anesthetics. The ‘inert’ anesthetic gas, xenon, was found in cultured neurons to be essentially inactive on GABA-activated currents but very effective to inhibit NMDA receptors (Franks et al., 1998). Similarly, effects of nitrous oxide, on the GABA receptor are minimal or inconsistent (Mennerick et al., 1998), but, like known NMDA receptor antagonists, inhibits both NMDA-mediated ionic currents and neurodegeneration (Jevtović-Todorvić et al., 1998). We now report that, at one atmosphere, xenon and nitrous oxide, but not nitrogen, bind within the *P*450 heme cavity, an occupation linked to perturbation of the catalytic activity of *P*450 enzymes.

With liver microsomal suspensions and each gas at 100% concentration, an absorbance-difference spectrum (Fig. 1) was generated by xenon (ED_{50} ~ 1.6 atm), nitrous oxide (ED_{50} ~ 1.5 atm) and 1,2-dichlorotetrafluorethane (ED_{50} ~ 1.0 atm), but not by nitrogen (ED_{50} ~ 50 atm). At concentrations near those that effect anesthesia, xenon, nitrous oxide or 1,2-dichlorotetrafluorethane, and each of the non-gaseous anesthetics studied previously, all yield quantitatively similar changes in absorbance. Accordingly, for all compounds that have been examined, the concentra-

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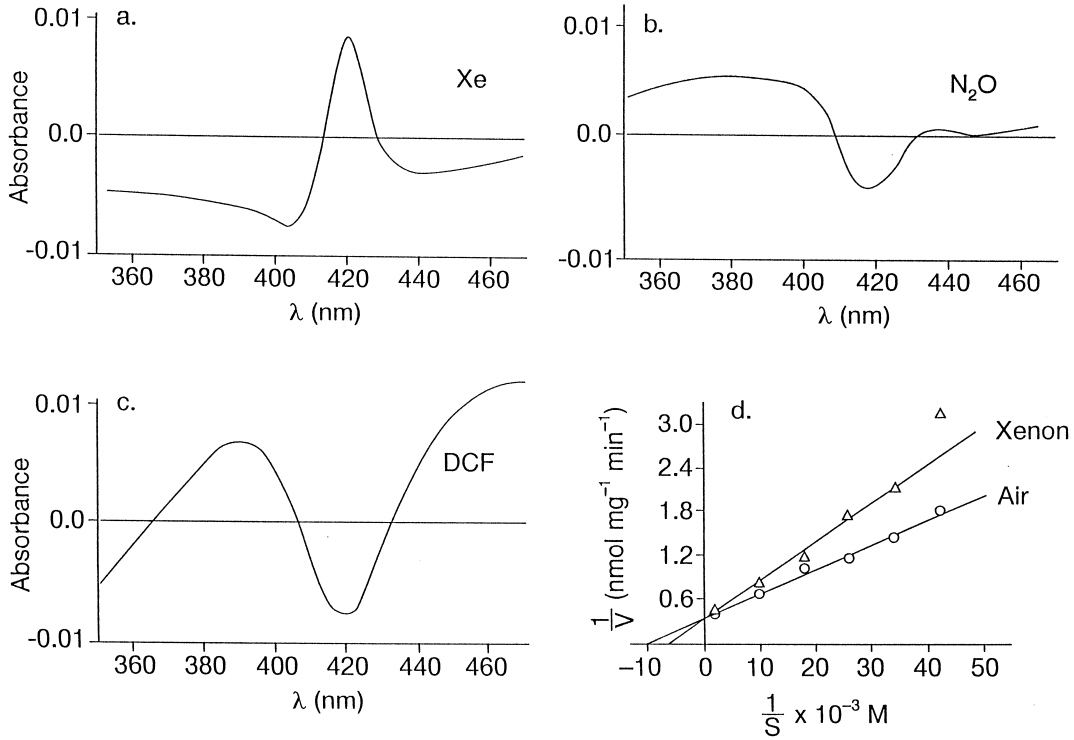

Fig. 1. An 'absorbance-difference spectrum' is obtained when a substance is added to a suspension of liver microsomes, with a reference cuvette containing microsomes only. The spectrum generated reflects the change in spin state of the iron atom in the *P*450 heme cavity. Virtually, all chemical compounds that are inhibitors of, or competing substrates for, cytochromes *P*450 yield an absorbance-difference spectrum and exhibit classical competitive inhibition of enzyme activity. Modification of the light absorbing properties of the heme moiety result from two major types of interaction: direct binding of ligand to the iron atom or perturbation of the heme moiety by the ligand at another site within the cavity. Microsomes from rat liver, 1 mg protein, were suspended in a 40-ml vial containing 1 ml Tris buffer that had been previously shaken in a pure helium atmosphere for 1 h to remove dissolved oxygen. The vial was flushed with helium and shaken for 2 h to remove other gases and enzyme-bound ligands, including oxygen; in the absence of this step, resolving an absorbance-difference spectrum by addition of a gas is confounded by displacement of any ligand already occupying the heme cavity. The helium in the test, but not the reference, flask was replaced by another pure gas and absorbance-difference spectra obtained. (a) Xenon, (b) nitrous oxide and (c) 1,2-dichlorotetrafluoroethane (see LaBella et al., 1998 for reproducibility of spectra). Lineweaver–Birk plot (d) shows a typical experiment showing competitive inhibition of microsomal aminopyrine demethylase by 80% xenon/20% oxygen; control tubes were incubated in air.

tions yielding similar absorbances are inhibitory to *P*450 catalytic activity. Xenon, like compounds in general that occupy the heme cavity, competitively inhibits cytochrome *P*450-mediated aminopyrine demethylase activity (Fig. 1d). The heme enzymes, prostaglandin synthase and nitric oxide synthase, and the non-heme, iron-containing lipoxygenase showed similar sensitivities to general anesthetics (unpublished; Chen and LaBella, 1997). The presumed relevance of the interaction of general anesthetics with heme proteins is perturbation of steady state levels of eicosanoids and other cognate chemical signals that determine cell sensitivity and response. A prominent role for eicosanoids generated in nervous tissue cells is modulation of the diverse complement of ion channels.

For any given agent, the concentrations that produce general anesthesia are not that dissimilar across the phylogenetic scale, leading to the presumption that only one or a few molecular species may constitute the underlying target mediators. Perhaps, interaction with only a single molecular species is the predominant determinant of the anesthetic state in all sensitive organisms. However, from the available data it appears almost certain that perturbation of multiple molecular mediators of anesthetic action, e.g., GABA receptor, NMDA receptor, *P*450 monooxygenases, gives rise to the well recognized, anesthetic-specific constellation of physiological changes associated with the state of general anesthesia. In the case of heme proteins, the absorbance-difference spectrum verifies occupation by anesthetic of the catalytic cavity. It remains to be determined to what extent the effects of anesthetics on the GABA, NMDA or other sensitive ion channels result from direct interactions with channel proteins or are mediated through changes produced by anesthetics in the levels of second messengers. In this regard, it should be noted that activation by volatile anesthetics of potassium currents in isolated neurons is potently obtunded by inhibitors of cytochromes *P*450 (Lopes et al., 1998). Furthermore, the contribution of each putative biomolecular mediator to the anesthetic state is unclear. For example, the $GABA_A$ receptor in rats appears to mediate immobility, but not amnesia/hypnosis, that is effected by volatile anesthetics (Quinlan et al., 1998).

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