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Title : THE SPIN-TRAPPING OF FREE RADICALS FORMED FROM HALOTHANE IN RAT LIVER

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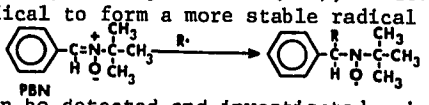
Introduction. Chemical compounds termed spin-trapping agents have recently been used to detect and identify reactive short-lived free radicals produced in biochemical systems. The spin-trapping compound, such as phenyl-t-butylnitrone (PBN), reacts with the free radical to form a more stable radical product:



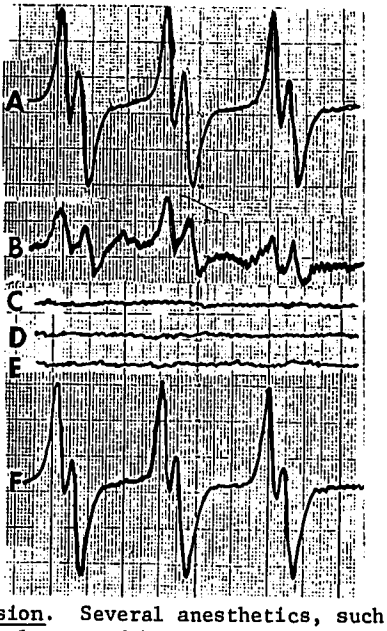
PBN

which can be detected and investigated using electron spin resonance (ESR) techniques[1]. This method has recently been employed to definitively demonstrate the formation of the $\cdot CCl_3$ radical from CCl_4, not only by a rat liver microsomal system[2], but also by the liver in vivo[3]. The results described here show that free radicals formed in vivo from the general clinical anesthetic, halothane, can be trapped using PBN, and isolated for detection by ESR spectroscopy.

Methods. The spin-trapping agent, PBN, was given at a dosage of 1 ml of a 0.14 M solution in 0.02 M phosphate buffer, pH 7.4. The buffered solution of spin-trap was homogenized with 1 ml of corn oil and administered as an emulsion by stomach tube after the rats had been fasted for 20 h. Immediately following the administration of the PBN-buffered-corn oil emulsion, halothane was given by inhalation at a dosage of 0.5% (v/v) in the inhalation air for 2 hours using a Foregger inhalator equipped with a Fluotec attachment. All rats were sacrificed 2 h after treatment and the livers immediately removed, and 5 g homogenized directly in chloroform-methanol (2:1). The extraction method of Folch et al was performed[4] and the $CHCl_3$ layer was evaporated in vacuuo to a volume of 1 ml or less. The concentrated extract was analyzed immediately in a Varian E-9 ESR spectrometer.

Results. Fig. 1 A shows the ESR analysis of a chloroform-methanol (2:1) extract of 5 g of liver from a rat which had been given halothane for 2 h following the oral administration of PBN. This reproducible ESR signal was obtained in every experiment of this type. Fig. 1 B shows the ESR spectrum obtained when halothane was incubated with rat liver microsomes and an NADPH-generating system. This ESR signal has the same characteristics as that from the in vivo system of Fig. 1 A. In this study the ESR signal was not seen in the lipid extract when fresh liver tissue from a rat treated with PBN and 10 ml of halothane were added simultaneously to the $CHCl_3$-CH_3OH extracting mixture (Fig. 1 C). Also, when the rats were given PBN but no halothane (Fig. 1 D), or were given halothane but no PBN (Fig. 1 E), no ESR signal could be seen in the liver lipid extracts. When the livers from rats given halothane and PBN were homogenized and fractionated into nuclei-plasma membranes, mitochondria, microsomes and the post microsomal soluble fraction, the lipid soluble material extracted and examined for an ESR signal, the extract from the microsomal fraction consistently showed an ESR signal (Fig. 1 F) and contained the major portion of the total trapped radicals in the liver homogenate.

Figure 1.



Discussion. Several anesthetics, such as halothane, are halogenated hydrocarbons, and are presumed to be metabolized in vivo by the NADPH-dependent drug metabolizing system in the endoplasmic reticulum of the liver. The control experiments shown in Fig. 1 C, D and E indicate that it is unlikely that the PBN-radical adduct is an artifact or is formed during the extraction and concentration of the liver lipid sample. The radical which is trapped in vivo appears to be identical to that formed by the metabolism of halothane by liver microsomes. This data provides evidence that halothane can be converted to a free radical in vivo in the endoplasmic reticulum of the liver.

References.

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