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SHORT COMMUNICATIONS

Effect of the volatile anesthetics on aniline hydroxylase and aminopyrine demethylase*

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IT IS a frequent occurrence in medicine to find a patient taking more than one medication and this is particularly true at the time of general anesthesia. Since metabolism is one means the biological system has of limiting the duration of action of a drug, one wonders what the effect of the presence of volatile anesthetics has on the metabolism of other drugs. Several of the volatile anesthetics and other organic solvents have been found to undergo metabolism by an enzyme system very similar to, or the same as, the hepatic mixed-function oxidase system[1] which carries out a large number of drug biotransformations. Therefore it is conceivable that an interaction between volatile anesthetics and other drugs may occur in the form of a competition between drugs for the enzymatic site. This communication reports the effect of certain volatile anesthetics on the metabolism of aniline and aminopyrine.

Microsomes were prepared from livers of male rats weighing approximately 200 g. The livers were perfused with cold 0·9% saline, removed, and then homogenized in cold 1·15% KCl. The final concentration of microsomes was adjusted to approximately 3 mg protein per ml of Tris buffer.

The formaldehyde produced by oxidative *N*-demethylation of aminopyrine was measured according to the method of Nash.[2] The standard reaction mixture contained 0·05 M Tris buffer, pH 7·5, microsomes (3 mg of protein), aminopyrine and an NADPH generating system. The latter consisted of 20 μmoles of glucose 6-phosphate, 1 μmole of NADP and 1 unit of glucose 6-phosphodehydrogenase. The concentration of aminopyrine used was 5·0 mM. Incubations were carried out for 20 min at 37°.

The *p*-aminophenol produced by the hydroxylation of aniline was determined according to the method of Imai *et al.*[3] This was run using the conditions described above for aminopyrine with the exception that aniline was used as substrate at a concentration of 1·0 mM.

The incubations were carried out in sealed flasks which were flushed with oxygen and 1 μl of the anesthetic or other organic solvent added just prior to sealing. The concentrations of anesthetic or other organic solvent in the incubation medium were determined at the conclusion of the incubation by extracting the medium with hexane. This extract was then chromatographed by gas chromatography using a bis-(2-ethylhexyl) sebacate on chromasorb column and hydrogen flame detectors. This was necessary since not all of the 1 μl of solvent added to the incubation medium went into solution and, thus, the amount added was not the same as the amount in solution. The enzyme assays and the quantitation of the organic solvents were parallel studies. Protein concentrations were determined by the method of Lowry *et al.*[4]

The results presented in Table 1 indicate that halothane, methoxyflurane and the trichloroethane isomers stimulate the aniline hydroxylase activity while there is no significant effect on the aminopyrine demethylase, under the conditions of substrate concentration employed. Anders[5] has reported that acetone enhances the aniline hydroxylase activity and Imai and Sato[6] have reported that ethyl isocyanide will also enhance this activity. This enhancement, which is similar to that reported in this communication, is not considered to be an uncovering of the hydroxylase enzyme but it is more likely an alteration of existing enzyme since Anders[5] has found that both the K_m and V_{max} are increased by acetone.

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TABLE 1. INFLUENCE OF VARIOUS ANESTHETICS AND ORGANIC SOLVENTS ON ANILINE HYDROXYLASE AND AMINOPYRINE DEMETHYLASE*

Additions to complete system†	Molar	Aniline hydroxylation (mμmoles/mg protein/min)	Formaldehyde formed from aminopyrine‡ (mμmoles/mg protein/min)
None		0·24 ± 0·04	1·20 ± 0·10
Methoxyflurane§	6×10^{-4}	0·42 ± 0·06‖	0·89 ± 0·38
Halothane¶	8×10^{-5}	0·36 ± 0·05‖	0·89 ± 0·30
Diethyl ether	5×10^{-5}	0·17 ± 0·10	0·62 ± 0·36
1,1,1-trichloroethane	1×10^{-4}	0·38 ± 0·05‖	1·40 ± 0·50
1,1,2-trichloroethane	1×10^{-4}	0·40 ± 0·06‖	1·20 ± 0·42
Chloroform	1×10^{-4}	0·24 ± 0·04	0·81 ± 0·40
Pentobarbital	1×10^{-4}	0·26 ± 0·04	1·18 ± 0·11

* Data presented as means (±S.E.M.) of four to six determinations.
† The anesthetics and organic solvents were added directly to incubation medium and their concentrations determined at the end of incubation period.
‡ Aniline hydroxylase was run according to the method of Imai *et al.*[3] The formaldehyde produced by demethylation of aminopyrine was determined by the method of Nash.[2]
§ 1,1-difluoro-2, 2-dichloroethylmethyl ether.
‖ Significantly different ($P = < 0{\cdot}01$) from control values (Students *t*-test). All other values not significantly different ($P = > 0{\cdot}05$) from controls.
¶ 1,1,1-trifluoro-2-chloro-2-bromoethane.

These findings are important not only from the point of view of the clinical situation and the problem of multiple drug therapy, but also in studies *in vitro*. Tissues are often removed from humans and animals during general anesthesia for the purpose of studying drug metabolism. Thus, care must be taken to make certain that the level of anesthetic is not high enough to influence the results of such assays *in vitro*. These studies are continuing in order to determine if other types of drug metabolism are effected by the presence of the volatile anesthetics.

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