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Is Depression of Mitochondrial Respiration a Predictor of In-vivo *Anesthetic Activity?*

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Previous data suggest that depression of mitochondrial respiration might be used as a predictor of *in-vivo* anesthetic activity. This hypothesis was tested by comparing the effects *in vivo* and *in vitro* of four volatile compounds with widely varying actions on the central nervous system. Caroxin-D and Caroxin-F, fluorocarbons devoid of anesthetic activity, were without effect on mitochondrial respiration. Hexafluoroisopropyl methyl ether (ISO), an inhalation anesthetic, depressed mitochondrial respiration in a fashion similar to that demonstrated for halothane, diethyl ether, methoxyflurane, enflurane, isoflurane, and fluroxene. These data support the above-mentioned hypothesis. However, flurothyl, an inhalation convulsant, also inhibited mitochondrial respiration. Moreover, while the effects of ISO and flurothyl are antagonistic *in vivo*, they were additive *in vitro*. It is concluded that the hypothesis that depression of mitochondrial respiration might be used as a predictor of *in-vivo* anesthetic activity is not valid. (Key words: Potency, anesthetic; Metabolism, mitochondrial; Theories of anesthesia; Pharmacology: fluorocarbons; Anesthetics, volatile: hexafluoroisopropyl methyl ether; Analeptics: flurothyl.)

HALOTHANE, diethyl ether, methoxyflurane, enflurane, isoflurane, and fluroxene produce dose-related inhibition of glutamate oxidation by rat liver mitochondria *in vitro*.[1-3] This occurs during state 3 respiration in the presence of exogenous adenosine diphosphate (ADP); state 4 respiration (exogenous ADP absent) is unaffected. The concentration of anesthetic necessary for 50 per cent inhibition of state 3 glutamate oxidation (ID_{50}) bears an inverse log-log relationship to lipid solubility as expressed by the oil–gas partition coefficient (O/G).[3] The minimum alveolar concentration of anesthetic necessary to prevent response to surgical stimulation in 50 per cent of patients (MAC) is an index of *in-vivo* anesthetic potency. MAC is also related to ID_{50}.[3] These data are consistent with a hypothesis that depression of mitochondrial respiration is a predictor of *in vivo* anesthetic activity.

The present study tests this hypothesis by examining the effects *in vitro* of four compounds having widely divergent effects on the central nervous system *in vivo*. Caroxin-D§ and Caroxin-F§ are fluorocarbons with considerable lipid solubility (table 1), but without effect on the central nervous system (J.H. Modell, University of Florida College of Medicine, Gainesville, Fla., personal communication). If depression of mitochondrial respiration is indeed a predictor of *in-vivo* anesthetic activity, these compounds should not affect mitochondrial respiration. In contrast, flurothyl (Indoklon, hexafluorodiethyl ether) is an inhalation convulsant, while its isomer, hexafluoroisopropyl methyl ether (ISO), is an inhalation anesthetic (table 1). These two compounds are antagonistic, and a mixture of 1.25 per cent ISO and 0.2 per cent flurothyl produces neither anesthesia nor convulsions in mice.[5] If the above hypothesis is valid, ISO should cause depression of mitochondrial respiration which can be antagonized by flurothyl.

Methods

The protocol for preparation of rat liver mitochondria, exposure to anesthetic vapors, and polarographic measurement (Oxygraph, Gilson Medical Electronics, Middleton, Wis.

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§ Trademark, Allied Chemical Corp., Morristown, N.J.

of state 3 and state 4 oxygen uptake with glutamate as substrate has appeared in a previous communication.[3] This method was modified for the four compounds investigated by use of a somewhat more complex anesthesia circuit (fig. 1). For each experiment, samples of mitochondrial suspension containing 29–44 mg protein/ml were placed in two test tubes maintained at 0–4 C in an ice bath. The control tube received 20 ml/min of air. Air was also directed to a primary and, for experiments utilizing mixtures of ISO and flurothyl, to a secondary vaporizer (dashed lines in fig. 1). The output of the vaporizers could be diluted with additional air. Excess vapor escaped through a pop-off valve; the remainder was allowed to flow at 20 ml/min through the tube containing the experimental preparation. Additional vapor or, when the vaporizers were bypassed, air alone could be directed to the oxygraph cuvette. This enabled equilibration of the reaction medium with vapor or air prior to measurement of oxygen uptake.

The vaporizers consisted of sidearm test tubes fitted with fritted glass gas dispersion tubes (# 5625-J16; A.H. Thomas Co., Philadelphia, Pa.). They were suspended in a constant-temperature water bath maintained at 30 C for Caroxin-D and Caroxin-F experiments and 25 C for experiments utilizing flurothyl and ISO. The flow of air through the vaporizers and that of diluent air added to reduce the concentration of vapor were metered through precision laboratory flowmeters (# 601, # 603; Matheson Gas Products, East Rutherford, N.J.). Use of these known

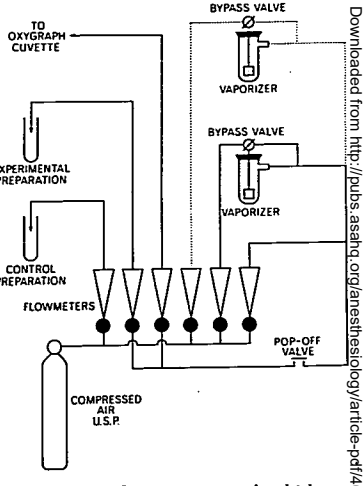

FIG. 1. Circuit for exposing mitochondrial suspensions to Caroxin-D, Caroxin-F, ISO, and flurothyl. The portion of the circuit denoted by dashed lines was used only when mitochondria were exposed to a mixture of ISO and flurothyl.

flows, the known ambient pressure, and the vapor pressure of each compound allowed calculation of the concentrations of each substance.

Most experiments with Caroxin-D or Caroxin-F utilized saturated vapor. For ISO and flurothyl experiments, several concentra-

TABLE 1. Characteristics of the Fluorocarbons*

	Caroxin-D	Caroxin-F	ISO	Flurothyl
Formula	$C_{10}F_{22}O_2$	$C_9F_{20}O$	$(CF_3)_2CHOCH_3$	$CF_3CH_2OCH_2CF_3$
Molecular weight	570	504	182	182
Vapor pressure (torr)	8.2 at 30C	20.5 at 30C	310 at 25C	202 at 25C
Lipid solubility (mg/g olive oil at 22 C)	0.93	1.17	—	—
Effect on central nervous system	None	None	Convulsant	Anesthetic

* Data were obtained from references 5 and 10 and J.H. Modell. The vapor pressure of flurothyl was calculated according to reference 11.

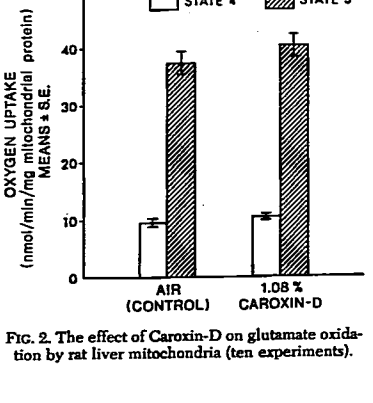

FIG. 2. The effect of Caroxin-D on glutamate oxidation by rat liver mitochondria (ten experiments).

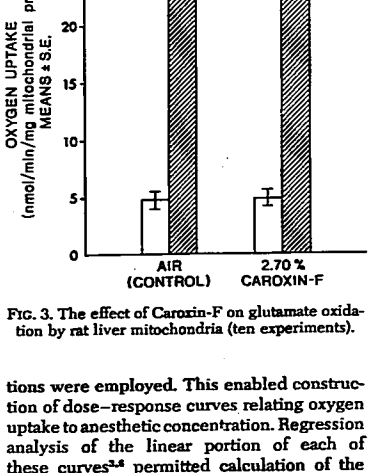

FIG. 3. The effect of Caroxin-F on glutamate oxidation by rat liver mitochondria (ten experiments).

tions were employed. This enabled construction of dose–response curves relating oxygen uptake to anesthetic concentration. Regression analysis of the linear portion of each of these curves[3,4] permitted calculation of the concentration of each agent which inhibited state 3 respiration by 25 per cent (ID_{25}). The regression of equations could also be used to predict the effect of any concentration of ISO or flurothyl on mitochondrial respiration. The interaction between ISO and flurothyl was examined in two studies. The first utilized a mixture of an ID_{25} of ISO plus an ID_{25} of flurothyl, predicted to be equipotent *in vitro*. The second examined the effects of the mixture of 1.25 per cent ISO plus 0.25 per cent flurothyl which Krantz *et al.*[5] found to produce neither anesthesia nor convulsions *in vivo*.

Results

The effects of saturated vapors of Caroxin-D and Caroxin-F on mitochondrial respiration are depicted in figures 2 and 3. Neither fluorocarbon affected state 3 or state 4 oxygen uptake. Although not shown, lower concentrations also did not depress state 3 or state 4 respiration.

Concentrations of ISO as great as 8.5 per cent did not affect state 4 oxygen uptake (fig. 4). However, there was dose-dependent depression of state 3 respiration (fig. 5) until a point at which higher concentrations had no further effect was reached. Regression analysis of the linear portion of the dose–response curve showed significant correlation between anesthetic concentration and oxygen uptake, with a predicted ID_{25} of 1.47 per cent. Flurothyl also had no effect on state 4 oxygen uptake (fig. 6). Again, however, there was dose-related inhibition of state 3 oxygen uptake (fig. 7). Calculations similar to those for the ISO data yielded a predicted ID_{25} of 2.86 per cent.

Exposure of mitochondria to the mixture of 1.48 per cent ISO and 2.86 per cent flurothyl produced 49.88 per cent inhibition of state 3 respiration (table 2). This figure agrees with the 50 per cent inhibition predicted if the effects of the two compounds were additive. The *in-vivo* equipotent mixture of 1.25 per cent ISO and 0.25 per cent flurothyl produced 25.70 per cent inhibition (table 3). On the basis of the two regression equations shown in figures 5 and 7, the predicted inhibition would be 23.39 per cent if the effects of the two compounds were additive.

Discussion

Observations as early as 1901[7–9] and as recently as 1967[4] suggest that lipid solubility may be used as a predictor of *in-vivo* anesthetic potency. While Caroxin-D and Caroxin-F have

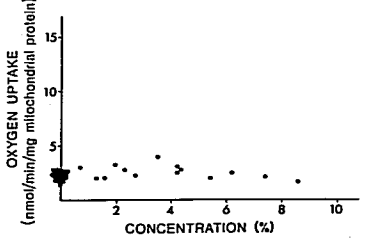

FIG. 4. The effect of ISO on state 4 glutamate oxidation by rat liver mitochondria. Sixteen experiments, each consisting of an experimental and a control preparation, were performed.

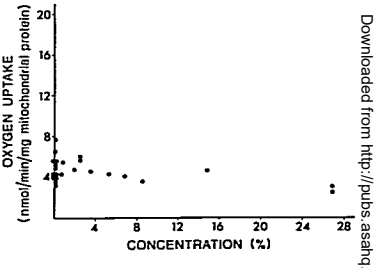

FIG. 6. The effect of flurothyl on state 4 glutamate oxidation by rat liver mitochondria. Twelve experiments, each consisting of an experimental and a control preparation, were performed.

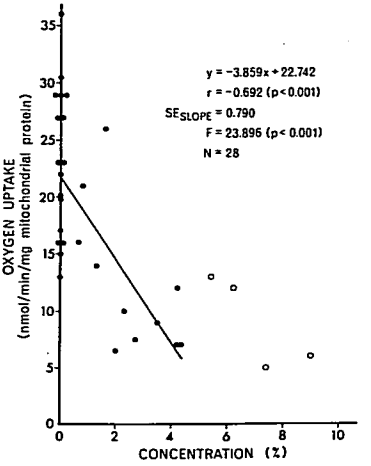

FIG. 5. The effect of ISO on state 3 glutamate oxidation by rat liver mitochondria. Sixteen experiments, each consisting of an experimental and a control preparation, were performed. Data indicated by open circles were not included in the regression analysis. These points are on the "plateau" portion of the dose–response curve and represent the lack of depression of the Amytal-insensitive pathway of electron transport.[3,12–14]

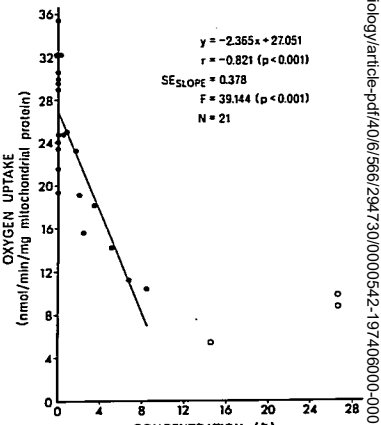

FIG. 7. The effect of flurothyl on state 3 glutamate oxidation by rat liver mitochondria. Twelve experiments, each consisting of an experimental and a control preparation, were performed. Data indicated by open circles were not included in the regression analysis, as in figure 5.

high lipid solubilities (table 1), they are devoid of anesthetic effects *in vivo* and *in vitro*. Thus, one cannot use lipid solubility as the sole determinant of anesthetic potency.

Can the effect of a volatile agent on mitochondrial respiration predict the *in-vivo* action? Data from previous studies[1–3] would suggest an affirmative answer. The present investigation indicates that both the nonanesthetic properties of the Caroxins and the anesthetic action of ISO would have been predicted correctly. However, the convulsant

TABLE 2. Effect of an *In-vitro* Equipotent Mixture of ISO and Flurothyl on State 3 Glutamate Oxidation by Rat Liver Mitochondria (Six Experiments)

ISO Concentration (Per Cent ± SE)	Flurothyl Concentration (Per Cent ± SE)	Oxygen Uptake (nmol/min/mg Mitochondrial Protein ± SE)	Per Cent Inhibition
None (control) 1.48 ± 0.01	*None (control)* 2.86 ± 0.02	21.45 ± 0.73 10.75 ± 1.69*	— 49.88

* Different from control ($P < 0.001$) when compared by Student's t test for paired data.[6]

TABLE 3. Effect of an *In-vivo* Equipotent Mixture of ISO and Flurothyl on State 3 Glutamate Oxidation by Rat Liver Mitochondria (Six Experiments)

ISO Concentration (Per Cent ± SE)	Flurothyl Concentration (Per Cent ± SE)	Oxygen Uptake (nmol/min/mg Mitochondrial Protein ± SE)	Per Cent Inhibition
None (control) 1.25 ± 0.06	*None (control)* 0.25 ± 0.01	25.37 ± 1.15 18.85 ± 2.31*	— 25.70

* Different from control ($P < 0.01$) when compared by Student's t test for paired data.[6]

flurothyl also produced dose-related inhibition of oxygen uptake. Furthermore, the effects of both the *in-vivo* and the *in-vitro* equipotent mixtures of flurothyl and ISO were not antagonistic, but were simply additive. Results of these experiments were not analogous to the effects observed *in vivo*.

N-pentane and n-hexane produce dose-dependent depression of mitochondrial respiration; administration of these hydrocarbons to rats is accompanied by convulsions. The ratio *in-vitro* potency/*in-vivo* potency is similar to that observed with the other centrally-acting drugs we have studied (unpublished observations).

Therefore, the use of depression of mitochondrial respiration as a *specific* model of anesthetic activity cannot be justified. Although only a few convulsant drugs have been studied, data at hand suggest that the hypothesis should be modified to include both *in-vivo* anesthetic and convulsant activity.

Samples of Caroxin-D and Caroxin-F were kindly supplied by Dr. Jerome Modell. Determinations of the lipid solubilities of these compounds were carried out in his laboratory. Flurothyl and ISO were a gift of Mr. James Vitcha, Ohio Medical Products, Murray Hill, N.J. 07974.

References

1. Cohen PJ, McIntyre R: The effects of general anesthesia on respiratory control and oxygen consumption of rat liver mitochondria, Cellular Biology and Toxicity of Anesthetics. Edited by BR Fink. Baltimore, Williams and Wilkins, 1972, pp 109–137
2. Cohen PJ, Marshall BE: Effects of halothane on respiratory control and oxygen consumption of rat liver mitochondria, Toxicity of Anesthetics. Edited by BR Fink. Baltimore, Williams and Wilkins, 1968, pp 24–36
3. Nahrwold ML, Cohen PJ: The effects of Forane and fluroxene on mitochondrial respiration: Correlation with lipid solubility and *in vivo* potency. ANESTHESIOLOGY 38:437–444, 1973
4. Saidman LJ, Eger EI II, Munson ES, et al: Minimum alveolar concentrations of methoxyflurane, halothane, ether and cyclopropane in man: Correlation with theories of anesthesia. ANESTHESIOLOGY 28:994–1002, 1967
5. Krantz JC Jr, Rudo FG, Loecher CK: Pharmacologic and physicochemical comparison of flurothyl and its isomer. Proc Soc Exp Biol Med 124:820–822, 1967
6. Batson HC: An Introduction to Statistics in the Medical Sciences. Minneapolis, Burgess, 1956
7. Meyer KH, Gottlieb-Billroth H: Theorie der narkose durch inhalations-anästhetika, Hoppe Seylers Z Physiol Chem 112:55–79, 1921
8. Meyer KH, Hemmi H: Beiträge zur theorie der narkose. Biochem Z 277:39–71, 1935
9. Overton E: Studien über die Narkose zugleich ein Beitrag zur allgemeinen Pharmakologie. Jena, G. Fischer, 1901
10. Saga S, Modell JH, Calderwood HW, et al: Pulmonary function after ventilation with fluorocarbon liquid P-12F (Caroxin-F). J Appl Physiol 34:160–164, 1973
11. Nahrwold ML, Archer PG, Cohen PJ: Estimation of an equation relating saturated vapor pressure to temperature. ANESTHESIOLOGY 39:444–446, 1973
12. Ernster L: Organization of mitochondrial DPN-linked systems. II. Regulation of alternative electron transfer pathways. Exp Cell Res 10: 721–732, 1956
13. Ernster L, Dallner G, Azzone GF: Differential effects of rotenone and Amytal on mitochondrial electron and energy transfer. J Biol Chem 238:1124–1131, 1963
14. Ernster L, Löw H, Lindberg O: The action of 5-ethyl-5-*iso*amylbarbiturate (Amytal) on the oxidation of reduced diphosphophyridine nucleotide (DPNH) in rat liver mitochondria. Acta Chem Scand 9:200–201, 1955