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Enzyme Induction by Enflurane in Man

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Concentrations of 6-β-hydroxycortisol (6-OHF), a polar metabolite of cortisol formed in the endoplasmic reticulum (microsomes) of the liver, and 17-hydroxycorticosteroids (17-OHCS) were measured in the urines of six healthy adult male volunteers exposed to a mean of 9.6 MAC-hours of enflurane anesthesia as an index of possible enzyme induction. The ratio of 6-OHF to 17-OHCS in 24-hour urine specimens collected five days before anesthesia was compared with the ratio of these metabolites in 24-hour urine specimens collected 16 to 18 hours after anesthesia. The ratio of 6-OHF to 17-OHCS increased markedly in five and decreased slightly in one volunteer following anesthesia. The results indicate that enflurane may cause induction of hepatic microsomal enzymes. (Key words: Anesthetics, volatile, enflurane; Biotransformation (drug), enflurane; Induction, enzymes.)

HEPATIC AND RENAL TOXICITY may be related to metabolic end product(s) or intermediate(s) formed during biodegradation of anesthetics.[1] The enzyme systems responsible for the formation of these products appear to be located principally in the endoplasmic reticulum (microsomes) of the liver. Enzyme induction can enhance the formation of the products of biodegradation.[2] However, there are no data available to indicate whether a single exposure to an anesthetizing concentration of a volatile anesthetic induces hepatic microsomal drug-metabolizing enzymes in man. To demonstrate enzyme induction by a volatile anesthetic in man requires 1) a relatively innocuous yet accurate means of defining the existence of induction of the hepatic microsomal drug-metabolizing enzymes, and 2) assurance that extraneous factors, *e.g.*, surgical stress or concomitant administration of other drugs, did not cause enzyme induction.

In man, enzyme induction may be suggested by demonstrating that: 1) the rate of clearance of a xenobiotic from plasma is greater; 2) its half-life in plasma is less; or 3) the excretion of its metabolite is increased after treatment with the suspected inducing agent. Enzyme induction in man may also be tested by a noninvasive method involving measurement of urinary levels of a metabolite of cortisol, 6β-hydroxycortisol (6-OHF) relative to the total of 17-hydroxycorticosteroids (17-OHCS). The test is based on the observation that drugs inducing hepatic microsomal drug metabolizing enzymes also induce hydroxylation of steroid hormones.[3,4] An increased excretion of 6-OHF relative to the total 17-OHCS following drug treatment is an indication of induction of hepatic microsomal hydroxylase and an indirect index of enzyme induction by drugs in man.[3]

The purpose of the present study was to determine whether a single exposure to an anesthetizing concentration of enflurane causes enzyme induction in man. We used as evidence of enzyme induction the ratio of the urinary levels of 6-OHF to the urinary levels of total 17-OHCS. We avoided extraneous factors such as stress of surgery and the concomitant administration of other drugs through the use of volunteers.

Materials and Methods

Six healthy male volunteers (ages 21–26 years) who had given informed consent and

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who were participants in an experiment designed to characterize the effects of enflurane on the cardiovascular, respiratory, renal and central nervous systems were studied.** Monitoring of these volunteers during anesthesia as well as the procedures used for induction, maintenance and recovery from anesthesia have been described.†† The subjects had not taken drugs known to affect drug metabolism, nor did they smoke tobacco or marihuana. No attention was given to the volunteers' dietary habits except to request that they not ingest alcoholic beverages for at least three weeks before entering the study. A 24-hour urine specimen was collected five days before anesthesia. The urine was refrigerated after each voiding and frozen upon completion of each 4-hour specimen. A second 24-hour urine specimen, begun 16 to 18 hours following 9.6 ± 0.4 SEM MAC-hours of enflurane anesthesia, was collected in a similar manner. Completeness of urine collections was checked by creatinine determinations. The concentrations of 6-OHF and 17-OHCS in the first 24-hour specimens served as the controls. The significance of the changes in the ratio of 6-OHF to 17-OHCS before and after anesthesia was evaluated by the t test for paired data.

6-OHF and 17-OHCS Determinations in Human Urine

Equal samples of a 24-hour urine specimen were extracted twice with equal volumes of ethyl acetate. The extract, after being washed twice with 0.1 N sodium hydroxide and twice with distilled water, was evaporated to dryness under a stream of nitrogen. The dried extract was dissolved in an ethyl acetate–methanol mixture and applied to precoated thin-layer plates (Brinkman). Standards of cortisol and 6-OHF were applied as markers in separate channels and the plates developed in a mixture of ethyl acetate: ethylene dichloride:water (90:10:1). Standards were located by ultraviolet scanning and the samples corresponding to 6-OHF were scraped from the plates and eluted with methanol. Following evaporation of the methanol, 6-OHF was quantitated against standard 6-OHF by the Porter-Silber[5] colorimetric procedure. Known quantities of 6-OHF were carried through each assay procedure and quantitated for correction of losses. Recoveries throughout the study averaged 40 per cent, and are reported uncorrected for losses.

Analysis of total urinary 17-OHCS involved incubation with beta-glucuronidase, extraction with ethyl acetate, washes in 0.1 N sodium hydroxide, and water evaporation under vacuum.[6] Quantitation was accomplished by the Porter-Silber method[5] utilizing ethanol, phenylhydrazine and sulfuric acid. The technique was modified from that previously described[6] only in the use of ethyl acetate rather than chloroform for extraction.

Results

As shown in table 1, five of the six volunteers had a marked elevations in the urinary excretion of 6-OHF following enflurane anesthesia. Anesthesia did not significantly change the urinary levels of 17-OHCS. One volunteer (Subject 5) had a 32.3 per cent increase in his output of 17-OHCS after anesthesia, but during the same period his excretion of 6-OHF increased by 296 per cent. Since 6-OHF is included in the total 17-OHCS, a more useful value in assessing the metabolism of cortisol is the ratio of 6-OHF to 17-OHCS in urine.[3] The percentage changes in the ratio of 6-OHF to total 17-OHCS were markedly increased in five and slightly decreased in one volunteer following anesthesia (table 2). The mean difference between the ratios of 6-OHF to 17-OHCS before and after enflurane anesthesia was significant, $P < 0.05$.

Discussion

Failure to find a significant increase in the urinary levels of total 17-OHCS (an index of adrenal cortisol output[7]) in five of the six volunteers following anesthesia suggests that exposure to 9.6 MAC-hours of enflurane vapor

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TABLE 1. Urinary 6-OHF and 17-OHCS before and after Enflurane Anesthesia

Subject	6-OHF (μg/24 hr)				17-OHCS (mg/24 hr)			
	Before	After	Difference	Per Cent Change	Before	After	Difference	Per Cent Change
1	23	53	30	+130	6.89	6.43	−.46	−6.6
2	48	194	146	+304	9.39	9.70	.31	+3.3
3	9	66	57	+633	6.05	6.09	.04	+0.6
4	8	7	−1	−12.5	2.91	3.19	.28	+9.6
5	27	107	80	+296	2.13	2.82	0.69	+32.3
6	41	110	69	+168	2.81	3.11	0.30	+10.6

was not a particularly stressful experience. However, one volunteer (Subject 5, table 1) had a significant increase in total 17-OHCS and 6-OHF following anesthesia. The disproportionately elevated percentage increase in this volunteer's excretion of 6-OHF relative to the percentage increase in excretion of 17-OHCS after anesthesia suggests that in this volunteer exposure to enflurane vapors not only enhanced adrenal output of cortisol but also stimulated the formation of 6-OHF.

A wide range of values for the urinary excretion of 6-OHF in normal healthy men has been reported.[4,6,8,10–16] Our results are in agreement with those reports. This variability among individuals makes it impossible to compare the urinary excretion of 6-OHF of one individual with that of another. However, by calculating the difference between the ratios of 6-OHF to 17-OHCS in urine excreted before and after anesthesia by the same individual, we were able to demonstrate that enflurane anesthesia markedly increased the metabolism of cortisol to 6-OHF in five of the six volunteers.

TABLE 2. Ratio of 6-OHF to 17-OHCS before and after Enflurane Anesthesia

	Before	After	Difference	Per Cent Change
Subject 1	.0036	.0082	+.0046	+127
Subject 2	.0051	.0200	+.0149	+196
Subject 3	.0015	.0108	+.0093	+666
Subject 4	.0027	.0022	−.0005	−19
Subject 5	.0127	.0379	+.0252	+198
Subject 6	.0146	.0354	+.0208	+142
			MEAN .0124 SD = .0098 $P < .05$	

Although the percentage changes in the urinary levels of 6-OHF following enflurane anesthesia were significantly increased in five of the six volunteers (table 1), the absolute values of 6-OHF both before and after anesthesia were lower than values we previously found in human volunteers before and after treatment with pentobarbital.[8] This may be explained by differences in the methods used to isolate 6-OHF. In our previous study, we used paper chromatographic procedures that required two days for isolation of 6-OHF; in the present study we employed a more rapid (45 min) thin-layer chromatographic procedure that significantly enhanced loss of 6-OHF.

The enzyme systems involved in the inactivation of cortisol are confined almost exclusively to the liver.[9] As shown in figure 1, a major pathway for catabolism of cortisol is its reduction to dihydrocortisol. This conversion, catalyzed by Δ^4-hydrogenase, involves the saturation of the double bond in ring A by the introduction of two hydrogen atoms at the 4-5 carbon linkage. The addition of two hydrogen atoms to the ketonic group at carbon 3 of dihydrocortisol by 3α-hydroxysteroid dehydrogenase results in the formation of tetrahydrocortisol (THF). Cortisone, which is formed from the oxidation of cortisol by 11β-hydroxysteroid dehydrogenase, can undergo further reduction to produce tetrahydrocortisone (THE). THF and THE can be further metabolized to cortol and cortolone, respectively, by an enzymatic process involving the addition of two hydrogen atoms to the ketonic group at carbon 20. Conversion of the 20 ketonic group of cortisol and cortisone to the 20 hydroxyl group can precede ring A reduction. The 17-keto derivative of cortisol

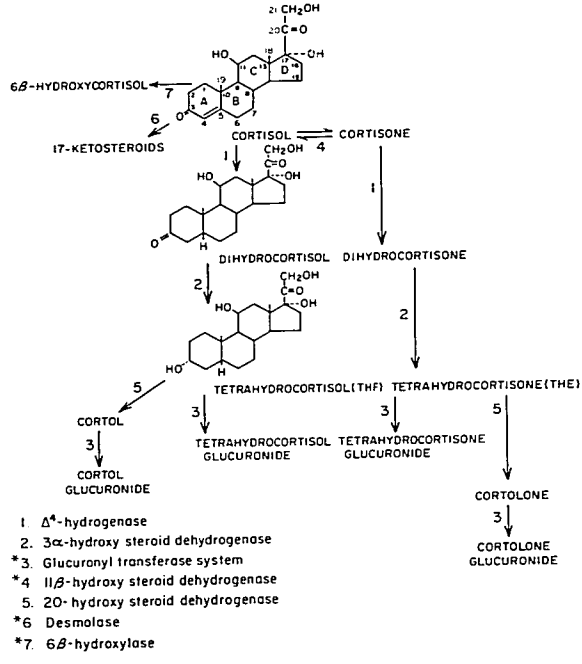

FIG. 1. Steps in the catabolism of cortisol by the liver. * = Enzymes localized in the endoplasmic reticulum (microsomes). Δ^4-hydrogenase exists as 5α- in the microsomes and as 5β- in the soluble fraction of liver homogenates; most of the reduced urinary metabolites of cortisol are in the 5β- form. 3α-hydroxysteroid dehydrogenase is associated with the supernatant fraction of liver homogenates. 20-hydroxysteroid dehydrogenase exists as 20α- in the cytosol and as 20β- in the microsomes.

is formed from the cleavage of 17-20 carbon linkage by a desmolase. In normal man, 6β-hydroxylation is a minor metabolic transformation. However, in the newborn and during pregnancy,[10] and following the administration of drugs that induce the hepatic microsomal drug-metabolizing enzymes,[3,4,8,13–16] the formation of 6-OHF is markedly increased.

The stimulatory effect of enflurane on cortisol hydroxylation reported here is similar to the effects of other hepatic microsomal enzyme inducers on the metabolism of cortisol in man.[3,4,8,13–16] Several of the drugs that enhance urinary excretion of 6-OHF in man have been shown to induce the enzyme system in guinea-pig hepatic microsomes that hydroxylates cortisol in the 6β position.[3,14] The increase in the ratio of 6-OHF to 17-OHCS following enflurance anesthesia could be accounted for by enzyme induction.

6-OHF, because of its water solubility, can clear the kidney without conjugation. In contrast, THF and THE, the major metabolites of cortisol, are conjugated with glucuronic acid to make them water-soluble (fig. 1). The glucuronides of THF and THE, which can easily

clear the kidney, represent about 30 per cent of the cortisol secretory rate, and are the major components of urinary 17-OHCS, the measurement of which serves as a valuable index of cortisol secretion.[7]

An alternate explanation for an increase in the urinary excretion of 6-OHF might be the inhibition of hepatic microsomal Δ^4-hydrogenase by enflurane, with a resultant increase in the amount of cortisol metabolized to 6-OHF. This explanation seems unlikely in view of our observation demonstrating no significant decrease in the production of 17-OHCS following enflurane anesthesia.

The observation that the urinary ratio of 6-OHF to 17-OHCS was slightly decreased following enflurane anesthesia in one volunteer suggests that there is individual variation in the susceptibility to enzyme induction by enflurane. Whether this variability is due to genetic and/or environmental factors cannot be answered by the present study. Both factors play significant roles in determining individual differences in the rates of metabolism of many substances that are substrates for the hepatic microsomal enzymes.[17,18]

The results reported here demonstrate that a single prolonged exposure to an anesthetizing concentration of enflurane significantly increased the urinary ratio of 6-OHF to 17-OHCS in man. This increase probably reflects hepatic microsomal enzyme induction.

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