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EFFECT OF HALOTHANE INHALATION ON OROTIC ACID UPTAKE AND URIDINE INCORPORATION INTO MOUSE LIVER RNA

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Abstract—Repeated exposure of mice to an oxygen–halothane atmosphere (99.1, v/v) caused the liver wt and RNA content to increase about 30 per cent. When RNA was labeled with radioactive orotic acid during this time, the labeling of RNA decreased about 50 per cent. The RNA precursor pool was not affected by halothane treatment. It could be demonstrated that the application of the anesthetic hampered the uptake of orotic acid from the blood into liver cells. Using radioactive uridine, this effect could not be observed. The study demonstrates that unexpected side effects of drugs might complicate the interpretation of results.

In vivo studies of RNA metabolism are usually performed by measuring the rate of specific precursor incorporation either into the bulk of cellular RNA or into specific species of RNA. In any case, the interpretation of such data is always difficult and might lead to serious pitfalls if a number of parameters are not carefully controlled.

The rate of a precursor incorporation can be modified by changes in the synthesis or the degradation of the RNA molecules; but more trivial explanations can account for those changes: for example, an alteration in the tissular uptake of the precursor, in the rate of its metabolism or also in the dimension of the endogenous precursor pool [3–5, 8, 10].

The administration of the widely used volatile anaesthetic halothane† causes in mice a rapid induction of the enzyme systems responsible for the transmitochondrial hydrogen transport [1, 2]. This effect is then followed by a considerable increase in both the liver weight and its RNA content. Nevertheless, at the same time, we observed an unexpected decrease in the labeling of total liver RNA when radioactive orotic acid was used as a precursor.

This observation led us to study the effect of halothane application on the metabolic fate of orotic acid and uridine, its *in vivo* metabolic products.

MATERIALS AND METHODS

Male NMRI mice (Ivanovas, Kissleg, Germany) of about 30 g were used throughout this study. The animals received food and water *ad libitum* and were exposed for 60 min daily (between 8 and 9 a.m.) to an oxygen–halothane (99:1, v/v) atmosphere produced by a Fluotec Marc II evaporator. The temperature was kept constant at 30° during anaesthesia.

The precursor incorporation experiments were usually performed 24 hr after the last halothane treatment. The animals received i.p. injections, either 0.25 μCi of [6-^{14}C]orotic acid (Amersham, sp. act. 55 Ci/mole)per g of body wt alone or for double labelling experiments, in a mixture with 2.5 μCi [5-3H]uridine (Amersham, sp. act. 550 Ci/mole). The labeled precursors were always administered in a 0.9% NaCl solution. RNA was routinely extracted at 65° from a liver homogenate in 5 vol. of phosphate buffer (0.155 M KH_2PO_4–0.0145 M Na_2HPO_4–0.14 M NaCl–0.5% SDS–100 μg/ml sodium heparine) by mixing with 1 vol. of water saturated phenol. The phenol phase was re-extracted as above. The combined water phases were then precipitated by the addition of 2 vol. of ethanol at −20° and the RNA collected 12 hr later by centrifugation.

To measure the free nucleotide fractions, the mouse livers were homogenized for 30 sec in 10 vol. of ice-cold 0.4 N $HClO_4$ with and Ultra Turrax (Type IP 18–10, Fa. Janke + Kunkel, Germany). After a 10 min centrifugation at 10,000 *g* the supernatant was neutralized with KOH 1 N and kept for 12 hr at 0–4°. After centrifugation, 3–4 ml of the supernatant (30–40 O.D. units at 260 nm) were chromatographed on a 30 × 0.9 cm PEI-cellulose column [6] which had been previously equilibrated with water. After removal of the non-absorbed materials by washing the column with water, the elution was further performed by a logarithmic 0–4.5 M NaCl gradient. The u.v. adsorption at 260 nm was recorded and the radioactivity of the collected fractions was measured in a Triton X-114 containing scintillation cocktail [7]. Identification of the different peaks was done by use of markers and the recording of u.v. spectra at different pH values.

The radioactivity in the serum of mice was measured as follows: After decapitation, the blood was collected in a heparine-containing physiological solution. After centrifugation, aliquots of the supernatant were counted in a Triton X-114 containing scintillation cocktail [7]. We verified by chromatography on PEI cellulose that the plasmatic radioactivity corresponded to orotic acid.

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†Halothane—$CF_3CHBrCl$.

Abbreviations—i.p.—Intraperitoneal injection, PEI-cellulose—polyethyleniminecellulose (Merck).

To measure the radioactivity incorporated into RNA, it was dissolved in 10 mM Tris acetate (pH 7.4) buffer and aliquots were precipitated by the addition of 5 vol. of cold 10% trichloracetic solution which contained $Na_4P_2O_7$ (10 mM). The precipitated RNA was collected on GF/F filter discs (Whatman) washed with cold 5% TCA (containing 20 mM $Na_2P_4O_7$) and with cold ethanol and finally hydrolyzed with 0.5 ml Hyamine 10-X (Packard) for 12 hr at 20°. Five ml of toluene based scintillation cocktail was then added to the digested solution and then counted.

The i.p. absorption of injected labeled orotic acid was verified as follows. Immediately after decapitation of the mice, the peritoneal cavity was injected with 5 ml isotonic NaCl solution which was then sucked out and injected again several times by the use of a syringe equipped with a needle. Finally, an aliquot of this same solution was diluted with Triton X-114 based scintillation cocktail and its radioactivity measured by liquid scintillation.

RESULTS

Daily 1-hr exposures to an atmosphere of 1 per cent halothane–oxygen is a very mild treatment, leading to only weak anaesthesia of the animals. Figure 1 illustrates the effects of such a treatment on the liver weight, its RNA content and the efficiency of the total liver RNA labeling by a 1 hr pulse with [6-^{14}C] orotic acid. The liver weight as well as its RNA content starts to increase after two to four daily 1-hr exposures of the animals to the anaesthetic and reached values corresponding to about 130 per cent of the control values after 6–8 inhalations. Thereafter, additional halothane applications do not further modify these two parameters.

In contrast, the incorporation of labeled orotic acid into total liver RNA was rapidly depressed and reached a value corresponding to about 50 per cent of the controls after four to five 1-hr halothane treatments. Both sp. act. of the RNA and total radioactivity incorporated in the liver RNA were decreased to about the same extent.

Table 1. Influence of single or repeated exposure of mice to halothane on the labeling of uridine nucleotides from mouse liver. Values are expressed as per cent of controls. Labeling was for 1 hr with 0.25 μCi [6-^{14}C] orotic acid/g body weight

Nucleotide fraction	dpm × 10^{-3} per g Liver control = 100% n = 12	% of control after one halothane treatment n = 4	% of control after seven halothane treatments n = 8
UTP	180 ± 21	70 ± 8	53 ± 8
UDP	169 ± 17	82 ± 4	67 ± 8
UMP + CMP	500 ± 47	89 ± 5	64 ± 12
UDP Sugar derivatives	790 ± 82	79 ± 7	58 ± 11
UDP Glucuronic acid	160 ± 15	75 ± 6	55 ± 7

To understand this apparent discrepancy in the results, we further analyzed the fate of the radioactive precursor in the animals. First, we studied the influence of the halothane treatment on the acid soluble nucleotides labeling after [6-^{14}C]orotic acid administration. In control animals, the different uridine containing compounds as well as CMP were rapidly labeled. A labeling of all these substances was strongly affected by the halothane treatment. As shown in Table 1, a single treatment was sufficient to significantly depress the radioactivity measured in all of the soluble nucleotides, and after seven successive exposures to the anaesthetic, the radioactivity dropped down to about 50–60 per cent of that measured in the control animals.

We then measured the pool size of the total acid extractable material and of the uridine nucleotide. The results presented in Table 2 clearly demonstrate that those parameters were not significantly modified by the halothane treatment.

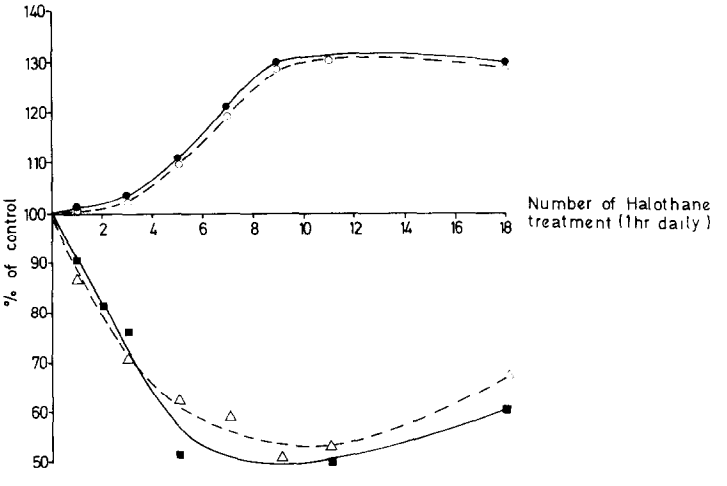

Fig. 1. Influence of Halothane treatment on liver wt, RNA content and labeling of total liver RNA. RNA was labeled for 1 hr by i.p. injection of 0.25 μCi [6-^{14}C] orotic acid/g body wt. Values are expressed as per cent of control—○---○— Liver wt, —●——●— RNA content, —△——△— sp. act. of total liver RNA, —■——■— DPM in total liver RNA, calculated for a 25 g standard animal.

Table 2. Effect of halothane treatment on pool sizes of total acid soluble material and uridine nucleotides from mouse liver

Number of halothane treatments	n	OD 260 nm per g liver: Total acid soluble material	UTP	UDP	UMP + CMP	UDP Sugar derivatives	UDP Glucuronic acid
None	12	5.7 ± 0.5	0.8 ± 0.5	0.5 ± 0.1	1.1 ± 0.1	2.3 ± 0.2	1.0 ± 0.2
One	4	5.9 ± 0.5	0.7 ± 0.1	0.5 ± 0.1	1.1 ± 0.2	2.3 ± 0.2	1.1 ± 0.1
Seven	8	5.5 ± 0.7	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.1	2.2 ± 0.3	1.0 ± 0.2

Table 3. Influence of halothane treatment on the recovery of radioactivity in serum of mice after different pulse times with [6-^{14}C] orotic acid. Serum from the mice was pooled for each experiment

Number of halothane treatments	Pulse time (min) with [6-^{14}C] orotic acid	dpm × 10^{-3} per ml of serum	Δ % Of control
None	15	58	—
	30	20	—
	60	12	—
One	15	78	+34
	30	31	+55
	60	18	+50
Seven	15	135	+130
	30	44	+126
	60	30	+150

To study whether the anaesthetic administration was modifying the uptake and/or the distribution of the RNA precursor, the fate of the injected orotic acid was systematically followed. Table 4 shows that the orotic acid was rapidly absorbed from the peritoneal cavity in both normal and treated mice. In parallel, the radioactivity was also measured in the blood of these animals (Table 3), here an important difference was found between the control and the halothane treated animals. In fact, the serum of the latter always contained a very significantly higher amount of radioactivity. A mixture of tritiated uridine and ^{14}C-labeled orotic acid was i.p. injected to the animals. Two hours later, the RNA specific radioactivity ([^{3}H] and [^{14}C]) was measured in the liver. Table 5 shows that the halothane treatment affected the RNA labeling by the two precursors differently. As expected, the incorporation of orotic acid was severely inhibited; no modification of the uridine incorporation was observed.

Table 4. Recovery of radioactivity in the peritoneum of mice at different intervals after the i.p. application of 0.25 μCi [6-^{14}C]orotic acid/g body weight. Values are calculated for 25 g standard mice

Time after an i.p. injection of labeled orotic acid (min)	Control: dpm in the peritoneum of a standard 25 g mouse $n = 4$	Control: % Recovery of applied orotic acid	Halothane (7 ×): dpm in the peritoneum of a standard 25 g mouse $n = 4$	Halothane (7 ×): % Recovery of applied orotic acid
15	93.656	0.63	90.280	0.61
30	66.600	0.48	66.610	0.48
60	22.200	0.16	28.458	0.2

Table 5. Influence of halothane treatment on the simultaneous labeling of mouse liver RNA with [5-^{3}H]uridine and [6-^{14}C]orotic acid. A mixture of 2.5 μCi [5-^{3}H]uridine and 0.25 μCi [6-^{14}C]orotic acid was applied per g body wt by an i.p. injection. Labeling was for 2 hr. Livers of three mice were pooled for each experiment

Halothane Treatment	Specific activity of RNA (dmp/mg): [^{3}H]	% Of control	[^{14}C]	% Of control
None	82.110	100	17.776	100
One	93.786	114	11.887	67
Seven	89.881	109	7.152	40

DISCUSSION

Halothane, a halogenated aliphatic hydrocarbon, widely used in human medicine, causes in the mouse an important increase in liver weight. In parallel, the mitochondrial and microsomal membranes proliferate quickly leading to a significant increase of both the RNA and protein liver content. When we started to study whether this increase in macromolecules could be directly attributed to changes in RNA

metabolism, we observed the paradoxical effect that the labelling of RNA was drastically decreased when radioactive orotic acid was used as a precursor. During the course of this study, we were able to demonstrate that the observed effect was not likely to be due to an impaired cellular uptake in the precursor.

It is known that the charges of the cell's surface influence the uptake of bromosulfothalein from the blood by the liver cell [9]. Like other anaesthetics, halothane might act with membrane constituents. Uridine and orotic acid are dissociated to different extents at blood pH values. An interaction of halothane with membrane constituents could then very well explain the selective effect of halothane on orotic acid uptake. However, to confirm this hypothesis, additional studies are necessary.

While all our experimental results strongly support the aforementioned hypothesis, there is still another less likely explanation to our results.

Due to the increased RNA synthesis, the neosynthesis of orotic acid and uridine might be strongly enhanced even though their pool size was not modified. As the enzymes involved in the transformation of orotic acid are very specific, it is possible that the newly made orotic acid would preferentially be used. The endogenous size of the orotic acid pool in the liver being very small, makes the precise measurement of its specific radioactivity difficult and consequently obliges us to retain this hypothesis as a valid possibility.

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