Studies of the Dual Effects of Halothane on the Lipolysis of Human Fat Cells

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Halothane has dual effects on lipolysis of human adipose tissue: at low tissue concentrations a stimulatory effect is found, while at higher tissue concentrations lipolysis is inhibited. The lipolytic response of human adipose tissue was studied in vitro with or without halothane, the phosphodiesterase inhibitor theophylline, the lipase activator dibutyryl cAMP (dbcAMP), the α-receptor antagonist phentolamine, the nonselective β-receptor antagonist propranolol, and the selective β₂-receptor antagonist practolol. In the absence of β-receptor antagonists low concentrations of halothane stimulated lipolysis. This effect was blunted by β-receptor antagonists, indicating that halothane at low tissue concentrations may directly stimulate the β-receptors. The inhibitory effect of higher tissue concentrations of halothane was not the result of increased α-receptor activity since addition of phentolamine did not inhibit this effect. High concentrations of theophylline or dbcAMP increased lipolysis in specimens exposed to halothane, but the lipolytic rate was still less than that found in specimens not exposed to halothane. The data thus indicate that the inhibitory effect of halothane is exerted at a step beyond the formation and degradation of cAMP. (Key words: Anesthetics, volatile, halothane; Metabolism, lipolysis; Metabolism, cAMP.)

Halothane is often accompanied by effects similar to those produced by stimulation of the β-adrenergic receptors e.g., vasodilation, bronchial relaxation and uterine hypotonia. Whether these effects are caused by direct β-adrenergic receptor stimulation is not yet clear. Klide et al.1 found that halothane relaxes smooth muscles from different sites in various species of animals and this effect is inhibited by β-receptor antagonists. Yang et al.2 on the other hand, found that halothane directly stimulates adenylate cyclase and phosphodiesterase activities in rat uterine muscle, an effect not inhibited by β-receptor antagonists.

The lipolytic process in human adipose tissue is mediated by the level of cyclic adenosine 3',5'-monophosphate (cAMP)3 induced by the β-receptor-adenylate-cyclase system.4 Mäkeläinen et al.5,6 found that halothane stimulates basal lipolysis in rat adipose tissue. We have found7 that halothane in low tissue concentrations stimulates lipolysis in human fat cells. However, at higher tissue concentrations halothane inhibits lipolysis.8

The present study was designed to clarify where halothane exerts its effects in the chain of events leading to breakdown of tissue triglycerides.

Material

Twenty patients (five men and 15 women) undergoing laparotomy were the subjects of the study. The patients had isolated abdominal disorders. Patients who had jaundice, malignancies, or endocrinologic disease were excluded. The age range was 27–68 years. Body weights ranged from 90 to 126 per cent of ideal weights.9 All patients fasted overnight before operation.

Methods

ANESTHETIC MANAGEMENT

The patients were premedicated with atropine alone, with promethazine and atropine, or with diazepam and atropine immediately before induction of anesthesia. Anesthesia was induced either with a short-acting barbiturate (thiopental or hexobarbital) or with fentanyl or droperidol, followed by succinyl-
choline for endotracheal intubation. Anesthesia was maintained using a circle system with nitrous oxide and oxygen. Soon after intubation a specimen of subcutaneous fatty tissue was taken. Halothane was not used before the tissue specimens were obtained.

**INCUBATION PROCEDURE IN VITRO**

The incubation procedure used has been described. Briefly, fragments of fatty tissue, weighing 25–50 mg, were incubated in 2.0 ml Krebs-Ringer bicarbonate buffer with albumin added at a concentration of 4 mg/ml (Bovine Albumin, Fraction V, Armour Pharmaceutical Co., Eastbourne, England), with or without propranolol or practolol (ICI, Macclesfield, England), phenolamine (CIBA, Basle, Switzerland), theophylline or dibutyryl cyclic adenosine 3',5'-monophosphate (dbcAMP) (Sigma Chemical Co., St. Louis, Mo.). The concentrations of the substances are given in the text.

Halothane was added either directly to the medium or via the gas phase above the medium. The concentrations used in the medium were $10^{-6}$, $10^{-5}$, $10^{-4}$, $10^{-3}$, and $10^{-2}$ M. The concentration of halothane in the gas phase was 0.5 per cent (v/v). The technique used with halothane in the gas phase is described elsewhere.

After addition of the appropriate substances, all incubations were performed in tightly stoppered glass vials for 2 h at 37°C at pH 7.4. The gas phase was 95 per cent O$_2$ and 5 per cent CO$_2$ with halothane in some experiments as discussed above. Samples of the incubation medium were taken for the determination of glycerol as described by Laurell and Tibbling. Tissue lipids were extracted with chloroform-methanol (2:1) as described by Folch et al. Glyceride-glycerol was determined in the chloroform phase according to the method of Carlson.

In accordance with the suggestion of several investigators, the metabolic data are expressed in terms of the cellularity of the tissue specimens. Mean cell diameter was determined as previously described. Mean cellular volume was calculated according to Goldrick and mean cellular weight as suggested by Hirsch and Gallian, assuming that the density of fat cells is that of triolein.

The numbers of fat cells in the specimens were then determined by dividing the triglyceride content by the mean cellular weight. Significance levels were calculated according to Student's $t$ test using an Olivetti table computer (Programma 101).

**Results**

**INHIBITORY EFFECT OF HALOTHANE ON LIPOLYSIS**

The inhibitory effect on the lipolysis of 0.5 per cent halothane in the gas phase above the medium was not overcome by the presence of phenolamine, an $\alpha$-receptor antagonist, at a concentration of $50 \mu$g/ml (table 1).

Inhibition of phosphodiesterase activity by theophylline or more direct stimulation of the lipase activity by dbcAMP in different concentrations increased lipolysis whether halothane was present or not (fig. 1). Statistically maximal effects were reached at a concentration of $10^{-3}$ M for both substances in the control group as well as in the specimens exposed to halothane. Tissue sensitivities to the two compounds were also the same since similar concentrations were required for half-maximal effects (means ± SEM 0.6 ± 0.2 $\times 10^{-3}$ M and 0.4 ± 0.1 $\times 10^{-3}$ M for theophylline and 0.7 ± 0.1 $\times 10^{-3}$ M and 0.7 ± 0.2 $\times 10^{-3}$ M for dbcAMP, respectively).

Halothane, 0.5 per cent, depressed basal lipolysis ($P < 0.025$) (fig. 1). This inhibitory effect was overcome by dbcAMP or theophylline. However, even in the presence of concentrations of these agents that in the absence of halothane produce maximal effects (fig. 1 and ref. 18), the lipolytic rates
HALOTHANE EFFECTS ON LIPOLYSIS

Fig. 1. Effects of different concentrations of theophylline and dibutyryl cyclic AMP (dbcAMP) on lipolysis in the absence (○) and in the presence (●) of 0.5 per cent (v/v) halothane. Results are means ± SEM for six incubations performed in duplicate.

of the specimens exposed to halothane did not reach those obtained with the same concentrations of dbcAMP and theophylline in the control group (fig. 1). Increasing the concentrations of theophylline and dbcAMP in the halothane-exposed group from 5 \times 10^{-3} \text{M} to 5 \times 10^{-2} \text{M} did not further increase lipolysis (glycerol release ± SEM, 222 \pm 33.2 to 203.8 ± 30.5 and 269.3 ± 42.8 to 265.2 ± 43.9 nmol/10^5 cells, respectively). In the presence of halothane theophylline-stimulated lipolysis was decreased compared with that without halothane at all concentrations used in both groups, i.e., to 5.0 \times 10^{-3} \text{M} (P < 0.05), while dbcAMP-stimulated lipolysis was decreased at concentrations of 10^{-4} \text{M} and 10^{-3} \text{M} (P < 0.05), with a tendency towards depression at 5 \times 10^{-3} \text{M} (0.05 < P < 0.1).

STIMULATORY EFFECT OF HALOTHANE ON LIPOLYSIS

In agreement with our previous findings, the addition of halothane in low concentrations (10^{-6} to 10^{-3} \text{M}) directly to the medium increased lipolysis (table 2).

This increment was abolished by the non-selective β-receptor antagonist propranolol at a concentration of 1 μg/ml. To characterize further the type of β-receptor involved, the selective β1-receptor antagonist practolol was used at a concentration of 50 μg/ml. Here, again, stimulation of lipolysis was abolished (table 2).

Discussion

Like other investigators in this field, we have used different ways to express the concentrations of halothane, depending upon the methods of administration. It should be clear, however, that the concentrations given are those added during the course of the experiment and not the actual tissue concentrations. However, for reasons previously discussed, higher tissue concentrations are reached with halothane in the gas phase.

Table 2. Effects of Propranolol and Prazosin on Lipolysis at Different Concentrations of Halothane

<table>
<thead>
<tr>
<th>Halothane (μM)</th>
<th>Glycerol Release (nmol/10^5 Cells)</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>0</td>
<td>103.2 ± 23.6</td>
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<tr>
<td>10^{-6}</td>
<td>130.2 ± 36.0</td>
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<tr>
<td>10^{-5}</td>
<td>130.4 ± 27.4</td>
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<tr>
<td>10^{-4}</td>
<td>123.7 ± 26.2</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>120.7 ± 34.4</td>
</tr>
<tr>
<td>10^{-2}</td>
<td>123.7 ± 32.5</td>
</tr>
</tbody>
</table>

* Halothane was added directly to the medium at the indicated concentrations. Results are means ± SEM of eight incubations performed in duplicate.

1 P < 0.05, no halothane vs. halothane added, significance determined by t-test for paired data.
The chain of events from the activation of adenylate cyclase via $\beta$-receptor stimulation or via other hormone membrane receptors to hydrolysis of triglycerides and liberation of glycerol and fatty acids is schematically shown in figure 2. Substances that have stimulatory or inhibitory effects upon lipolysis may exert their actions at different sites in the chain.

The stimulatory effect on lipolysis of low concentrations of halothane that we reported earlier, which was abolished by $\beta$-receptor antagonists. It thus seems that this lipolytic effect of halothane is exerted via direct $\beta$-receptor stimulation as indicated in figure 2. This agrees with the findings of Mäkeläinen et al.,5,6 in rat adipose tissue and those of Klüve et al.,1 studying various organs in different animals. Our data with a nonselective and with a selective $\beta$-receptor antagonist indicate that the receptors involved are $\beta_2$-receptors. This finding is in accord with other studies showing that lipolysis is mediated mainly via $\beta_2$-receptors,19 although it has been shown that $\beta_2$-receptors may also mediate a lipolytic response.20 The previously described inhibitory effects of halothane on lipolysis are reached with higher tissue concentrations. The inhibitory effect, however, would be more important than the stimulating action from a clinical point of view, at least in adipose tissue, since studies of specimens from the same patients before and after 30 minutes of exposure to halothane have shown that the lipolysis is inhibited by halothane.7

In the present investigation this inhibitory effect was studied with regard to possible $\alpha$-receptor stimulation or possible effects upon the actions of phosphodiesterase or cAMP. It is well documented that stimulation of the $\alpha$-receptors in human fat cells may lead to a decrease in the lipolytic activity.12,21 As the inhibitory effect of halothane was not al-
tered whether the α-receptor antagonist phenolamine was present or not, it is concluded that halothane exerts no α-stimulatory effect in human adipose tissue.

The inhibitory effect of halothane on basal lipolysis was overcome by both dbcAMP and theophylline. The lipolytic effect of dbcAMP is mediated by the activation of triglyceride lipase. However, in the presence of both halothane and dbcAMP at all concentrations used, lipolysis was depressed compared with controls. Similarly, there was a depression of lipolysis in the presence of halothane and theophylline. (Supra-)maximal concentrations of theophylline and dbcAMP were used both in the absence (fig. 1 and ref. 18) and in the presence of halothane. Thus, the present data do not indicate that the decreased lipolysis associated with halothane depends upon an increased activity of the enzyme phosphodiesterase, degrading cAMP to 3'-AMP, nor do they indicate that halothane competitively inhibits dbcAMP-stimulated lipolysis. The data indicate, therefore, that the inhibitory effect of halothane on lipolysis is not the result of decreased amounts of available cAMP. The inhibitory effect of halothane appears instead to be exerted at a step after formation of cAMP, e.g., activation and/or the effect of the lipase on the hydrolysis of the triglycerides. It is also possible, of course, that halothane exerts an effect on membrane permeability to glycerol and fatty acids, although this possibility seems less likely.

Halothane is a highly lipophilic substance with a solubility coefficient in oil of 220 (ref. 22). Tissues that have low lipid contents will therefore accumulate less halothane than lipid-rich tissues.23 That effects resembling those of β-adrenergic stimulation are frequently noticed during halothane anesthesia may be due to less accumulation in the less lipid-rich tissues. Low tissue concentrations of halothane appear to lead to a direct stimulation of the β-receptors, as shown in the present study. In lipid-rich tissues such as the adipose tissue, however, accumulation of halothane during anesthesia is adequate to lead to an inhibitory effect even when fairly low concentrations are given.8

The excellent technical assistance of Mrs U. Carlbrand and Mrs C. Goldmark is gratefully acknowledged.

References


Obstetric Anesthesia

LAMAZE PREPARATION AND NEONATAL STATUS “Prepared childbirth” offers obvious psychologic advantages to both parents. Are there also physiologic advantages for mother and child? The authors have evaluated the course of labor and delivery in 129 primiparas who had completed Lamaze training. They were compared with 129 matched controls not undergoing such training. The two groups were similar with respect to maternal age, fetal presentation, use of oxytocics, length of labor, and the responsible physician (staff vs. resident). There was a higher incidence of spontaneous vaginal delivery (63 per cent) in the Lamaze group compared with the controls (36 per cent). The use of low forceps was more frequent in the control patients (54 per cent) than in those with Lamaze training (25 per cent). The two groups were similar in the incidences of Cesarean section as well as ante- and postpartum complications. In the group with Lamaze training, 28 per cent required no anesthesia (general or conduction) during the first stage of labor. Only 7 per cent of the control group received no anesthesia. Epidural or caudal anesthesia was administered to 14 per cent of the Lamaze and 40 per cent of control patients. Narcotic analgesics were administered to 65 per cent of the Lamaze and 84 per cent of control patients. Of parturients requiring narcotics, the total dose was significantly lower in the Lamaze group. The two groups were similar in the incidences of intrapartum fetal distress, Apgar scores, and infant morbidity. (Scott JR, Rose NB: Effect of psychoprophylaxis (Lamaze preparation) on labor and delivery in primiparas. N Engl J Med 294: 1205–1207, 1976.) ABSTRACTOR’S COMMENT: It would be interesting to know whether newer methods of evaluation of the newborn’s neurobiologic status would have demonstrated differences between the two groups.