Anesthesiology 2003; 98:437–48

Thermogenesis Inhibition in Brown Adipocytes Is a Specific Property of Volatile Anesthetics

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Background: **This investigation examined the possibility that the inhibitory effect of halothane on nonshivering thermogenesis (heat production) in brown adipocytes is not a universal effect of all anesthetic agents but related to the type of anesthetic.**

Methods: **Brown adipocytes from hamster were isolated with a collagenase digestion method and incubated with anesthetic agents. The rate of oxygen consumption was measured with an oxygen electrode. The effect of clinically relevant (and higher) doses of anesthetics of different classes on basal and norepinephrine-induced thermogenesis (oxygen consumption) was tested.**

Results: **Two distinct groups of anesthetics could be distinguished: thermogenesis inhibitors and noninhibitors. Thermogenesis inhibitors include volatile anesthetics such as halothane (IC_{50}, 1.1 mM), ether (IC_{50}, 20 mM), and chloroform (IC_{50}, 2.2 mM) (nominal concentrations), but also tribromoethanol (IC_{50}, 0.6 mM), all inducing inhibition of norepinephrine-induced thermogenesis without affecting the EC_{50} for norepinephrine. Thermogenesis noninhibitors include the nonvolatile anesthetics pentobarbital, propofol, ketamine, and urethane, the inhalation anesthetic nitrous oxide, and, notably, also the volatile nonanesthetics (nonimmobilizers) 1,2-dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane; none of these compounds had any effect on norepinephrine-induced thermogenesis at any concentration tested.**

Conclusions: **There are two distinct classes of anesthetics with regard to effects on thermogenesis, thermogenesis inhibitors and thermogenesis noninhibitors. The results are important for the interpretation of studies in thermal biology in general; specifically, they indicate that conclusions concerning regulation of nonshivering thermogenesis during anesthesia depend on the type of anesthetic used. Of clinical importance is that the volatile anesthetics are inhibitory for nonshivering thermogenesis and thus for an alternative heat production when myorelaxants prevent shivering. As the distinction between thermogenesis inhibitors and thermogenesis noninhibitors corresponds to the distinction between volatile and nonvolatile anesthetics, it may be related to the mode of action of the volatile anesthetics.**

THE molecular mechanism of action of general anesthetics remains unresolved. Originally an idea of a unitary site and mode of action for all general anesthetics was predominant.[1,2] However, in recent years, this hypothesis has been questioned; the current opinion is that there may be selective modes of action for different types of anesthetic agents.[3,4]

For an understanding of the mechanism of action of volatile general anesthetics (with halothane as type compound), the fact that they possess a remarkable property additional to their anesthetic function may be of significance: they fully inhibit norepinephrine-induced, uncoupling protein-1–dependent[5] thermogenesis in brown adipocytes.[6] This inhibition is reversible, occurs within clinically relevant concentrations of the anesthetics, and is a property shared by at least the volatile anesthetics halothane, enflurane, and isoflurane.[6] The inhibition is not only observable in isolated brown adipocytes; it is also evident during anesthesia of experimental animals.[7] The possibility exists that the thermogenesis inhibitory action could be functionally related to the anesthetic mode of action of these compounds. Evidently, this raises the question whether the thermogenesis inhibitory action is a specific property of the group of volatile anesthetic compounds or if it is a general effect of all anesthetic agents. If it is specifically associated with the subgroup of volatile anesthetics, there may, at the molecular level, be similarities in the mode of action of these compounds as anesthetics and as inhibitors of thermogenesis. A distinction between different groups of anesthetics in this respect would thus support the notion that different groups of anesthetics possess different modes of action at the molecular level. In addition, concerning body temperature control during anesthesia, there are good reasons to clarify whether differences between anesthetic agents exist with respect to their possible interaction with brown adipose tissue–derived nonshivering thermogenesis. Knowledge of which anesthetics may attenuate thermogenic function in patients (especially neonates and infants) during anesthesia is clearly of importance from a clinical point of view. Therefore, an identification of anesthetics that are thermogenically inhibitory or noninhibitory may be of clinical value. To answer these questions, a systematic study of different types of anesthetics with respect to their potential antithermogenic properties was conducted.

Materials and Methods

Cell Preparation

This study was ethically approved by the Animal Ethics Committee (Stockholm North Region). For isolation of brown adipocytes, two to three adult Syrian hamsters (*Mesocricetus auratus*), females and males, were used in each experiment. The animals were kept at 18–20°C

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Received from the Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, Stockholm, Sweden; the Department of Anesthesiology and Intensive Care, Lund University Hospital, Lund, Sweden; and the Karolinska Hospital, Stockholm, Sweden. Submitted for publication May 28, 2002. Accepted for publication October 7, 2002. Supported by a grant from the Swedish Science Research Council, Stockholm, Sweden, and from AGA Gas AB, Sundbyberg, Sweden.

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with a light–dark cycle of 6–18 h with free access to water and food (sunflower seed, dried carrots, wheat, oats, and rabbit–guinea pig pellets). The animals were anesthetized with carbon dioxide and decapitated. The interscapular, cervical, and axillary brown adipose tissue depots were dissected out and cleaned. Brown adipocytes were isolated in Krebs-Ringer phosphate buffer with a collagenase digestion method described previously in detail,[6] with some minor modifications: collagenase type II (Sigma, St. Louis, MO) was used, the first incubation was with 1.3 mg/ml collagenase, the second was with 0.67 mg/ml, and, if necessary, a further incubation with 0.33 mg/ml was performed. The collagenase incubations were performed in a thermostated water bath at 37°C. After the last collagenase incubation, the cell suspension was washed twice. The cell suspension was stored on ice and was used for experiments on the preparation day. In the experiments, anesthetics were examined for their effect on basal and norepinephrine-induced oxygen consumption (thermogenesis) in the isolated brown adipocytes. The anesthetics were added as detailed in the next three sections.

Volatile Anesthetics in Solution

For the current study, where volatile and nonvolatile anesthetics are compared, it was desirable with an evaluation of the effect on heat production in brown adipocytes of all types of anesthetics, when they were added directly to the cell suspension in the oxygen chamber. The final concentrations of the compounds in the cell suspension were adjusted to cover clinically relevant concentrations, as indicated from the references quoted for each anesthetic, as well as concentrations higher than these. The free aqueous concentrations of halothane, ether, and chloroform corresponding to the minimum alveolar concentration (MAC) value are here referred to as MAC_{aq}.

Halothane (Fluothane®; Zeneca Ltd., Macclesfield, Cheshire, England) was diluted in dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) and added in 11 μl to the cell suspension; DMSO was used for the controls. In a simple chemical equilibration between halothane and a physiologic salt solution, 1% halothane corresponds to ≈0.33 mM halothane at 37°C,[8,9] and the MAC_{aq} for halothane has been calculated to be 250 μM.[10] However, in the current investigation, physiologic concentrations (4%, 0.6 mM) of albumin were present in the buffer. Each albumin molecule has at least three high-affinity halothane-binding sites[11] that should initially be unoccupied, as a fatty-acid-free albumin preparation was used. The free halothane concentration is thus probably markedly lower than the nominal one in the present experiments.

Ether (diethyl ether anhydrous; May and Baker Ltd., Dagenham, United Kingdom) solutions in water were prepared by thoroughly mixing 0.5 ml ether with 3 ml water in an airtight glass tube. From this saturated solution, ether was added to the cell suspension in the oxygen electrode chamber; water was used for the controls. The concentrations indicated are the nominal concentrations deduced from the saturated solutions according to The Merck Index, # 3751.[12] MAC_{aq} for ether is 13 mM.[13] Chloroform (chloroform pro analysis; Merck) solutions in water were prepared in the same way as the ether solutions; saturated solutions according to The Merck Index, # 2111.[12] MAC_{aq} for chloroform is 1.1 mM.[10]

Tribromoethanol (Avertin, 2,2,2-tribromoethanol; Aldrich, Gillingham, United Kingdom), although not being a volatile compound and mainly being used for injection, has close molecular similarities to, *e.g.*, halothane. It was therefore in this context placed in the same category as the volatile compounds. Tribromoethanol was dissolved and diluted in ethanol and added in 10 μl ethanol to the indicated concentrations; 10 μl ethanol was used for the controls. Plasma concentrations of tribromoethanol during anesthesia have not been reported; 350 mg/kg is the recommended dose for injection in rodents.[14]

Nonvolatile Anesthetics

Anesthetics that do not belong to the volatile anesthetics can readily be added directly, in solutions, to the suspension of isolated adipocytes.

Pentobarbital (Apoteket; Production and Laboratories, Stockholm, Sweden) was dissolved and diluted in water and added to the indicated concentrations in 10 μl; water was used for the controls. The free aqueous concentration of pentobarbital calculated to correspond to the MAC value, *i.e.,* MAC_{aq}, is 40–50 μM.[3,15] Propofol (Diprivan® 20 mg/ml; AstraZeneca) was directly drawn from the purchased solution in volumes of 0.5–11 μl; the solvent for propofol, *i.e.,* the fat emulsion Intralipid® 10% (Pharmacia AB, Stockholm, Sweden), was used for the controls. The free plasma concentration associated with the hypnotic effect of propofol (MAC_{aq}) has been calculated to be 0.4–1.5 μM.[3] However, as protein binding of propofol is very high (97.8%),[3] the total blood concentrations are much higher: more than 10 μg/ml (≈56 μM) have been found at onset of anesthesia and 4 μg/ml (≈25 μM) during continuous infusion.[16] As the buffer used in the current experiments contained 0.6 mM albumin, which binds propofol,[11] 56–1,100 μM propofol (corresponding to 10–200 μg/ml) as used here should amply cover clinically relevant concentrations, despite the binding of propofol to albumin. Ketamine (ketamine hydrochloride; a gift from Parke-Davis Scandinavia, Solna, Sweden) was dissolved and diluted in water and added to the indicated concentrations in 10 μl; water was used for the controls. The effective plasma concentration of ketamine[17] is 100–150 ng/ml (≈360–540 nM). Plasma concentrations of ketamine as high as 10 μM have been measured during anesthesia in humans.[18] Urethane (ethyl carbamate; Sigma) was dissolved and diluted in wa-

ter and added to the indicated concentrations in 10 μl; water was used for the controls. The plasma concentration of urethane for sedation in humans is about 6.5 mM,[19] and during anesthesia in rat, the blood concentration is 0.8 mg/ml (≈10 mM).[19]

Nitrous oxide (AGA Gas AB, Sundbyberg, Sweden) gas mixtures of 21% O_2 and 5% CO_2 with 74% N_2, 25% N_2O–49% N_2, 50% N_2O–24% N_2, or 74% N_2O were used for preincubation of the isolated brown adipocytes before measurements of oxygen consumption, in the same way as with halothane in gaseous form (see below). The MAC for nitrous oxide is about 110%. Only clinically relevant concentrations were tested here.

Ethanol (95% ethanol; Kemetyl AB, Haninge, Sweden), 5–30 μl, was directly added when the compound was examined *per se*. The concentration range tested here was defined from the observations that learned skills in humans are impaired at plasma concentrations of 8 mM ethanol and that the fatal concentration is about 87 mM.[20]

Volatile Nonanesthetics (Nonimmobilizers)

1,2-Dichlorohexafluorocyclobutane (Chemtronica; Lancaster Synthesis, Morecambe, United Kingdom) and 2,3-dichlorooctafluorobutane (Chemtronica; Lancaster Synthesis) are halogenated volatile compounds, denoted nonanesthetics or nonimmobilizers, which do not induce anesthesia[21] despite being structurally related to the volatile anesthetics. 1,2-Dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane were diluted in DMSO and added in 10 μl; 10 μl DMSO was used for the controls. From lipid–gas solubility data, "predicted anesthetic" concentrations—here referred to as MAC_{pred}—have been calculated for these chemicals: ≈21 μM for 1,2-dichlorohexafluorocyclobutane and ≈8 μM for 2,3-dichlorooctafluorobutane.[22,23]

Measurements of the Rate of Oxygen Consumption

All measurements were performed in a magnetically stirred and thermostated (37°C) oxygen electrode chamber, with the isolated brown adipocytes in a Krebs-Ringer bicarbonate buffer; composition: 145 mM Na^+, 6.0 mM K^+, 2.5 mM Ca^{2+}, 1.2 mM Mg^{2+}, 128 mM Cl^-, 1.2 mM SO_4^{2-}, 1.2 mM $H_2PO_4^-$, 25.3 mM HCO_3^-, 0.6 mM (4%) fatty-acid-free bovine serum albumin (Fraction V fatty acid free; Boehringer Mannheim, GmbH, Germany), 10 mM glucose, and 10 mM fructose. The buffer was bubbled with 5% CO_2 in synthetic air (21% O_2 and 79% N_2) at 37°C, and, when necessary, the pH was adjusted to 7.4 with HCl before cells from a concentrated cell suspension in Krebs-Ringer phosphate buffer were added to the buffer. An air-tight lid was adjusted to the chamber, and additions of norepinephrine [(-)-Arterenol; Sigma) were made through a small hole in the lid of the chamber. The rate of oxygen consumption was measured polarographically with a Yellow Springs Instrument 4004 Clark-type oxygen probe equipped with a YSI 5775 Standard 25-μm Teflon membrane (YSI Incorporated, Yellow Springs, OH).

The signal from Clark-type electrodes (as used in the current investigation) has been reported to be affected by halothane.[24,25] The equipment was therefore tested with halothane in DMSO or in buffer, and with buffer gassed with 4% halothane; no effect could be detected on the signal from the electrode (in accordance with tests performed by other investigators with this version of the Clark electrode[25]). In the description from the manufacturer, nitrous oxide is also included in the list of gases that can affect the signal from the electrode. The equipment was therefore tested with buffer bubbled with 74% nitrous oxide; no interference with the signal could be detected.

The output signal from the oxygen electrode amplifier was collected every 0.5 s by a MacLab/2e (application Chart v3.5; AD Instruments, Hastings, East Sussex, United Kingdom). The MacLab Chart file was transferred to the KaleidaGraph Macintosh application (Albeck Software, USA). After conversion of the data files to absolute values, they were used for calculations of mean rates of oxygen consumption (the running means) and for presentation of the experiments. The values used for conversion of the sampled values to the rates of oxygen consumption were an oxygen content of 217 nmol/ml, the calibrated electronic differentiator constant, the cell number, and the zero current value of the differentiated signal in each experiment.

Experimental Procedure

About 100,000 brown adipocytes in 1.1 ml Krebs-Ringer bicarbonate buffer bubbled with 5% CO_2 in synthetic air at 37°C were directly added to the oxygen electrode chamber (no preincubation). After 2 min, the indicated anesthetics or nonanesthetics were added and incubated with the cell suspension for 5 min in the closed oxygen chamber, before addition of norepinephrine. To allow for examination of alterations in norepinephrine sensitivity as well as in total thermogenic capacity as an effect of anesthetic agents, the basal rate of oxygen consumption was first registered; thereafter, increasing concentrations of norepinephrine were successively added.

Halothane in Gaseous Form

About 100,000 brown adipocytes were preincubated for 15 min in a temperature-controlled (37°C) shaking water bath in 1.4 ml Krebs-Ringer bicarbonate buffer with the indicated concentrations of halothane delivered *via* a conventional vaporizer. Carrier gas was synthetic air (AGA Gas AB) with 5% CO_2. Preincubations were performed with the double-needle technique described previously.[6] Halothane concentrations were continuously measured with a Servo Gas Monitor 120 (Siemens-Elema, Sundbyberg, Sweden) at the inlet of the preincu-

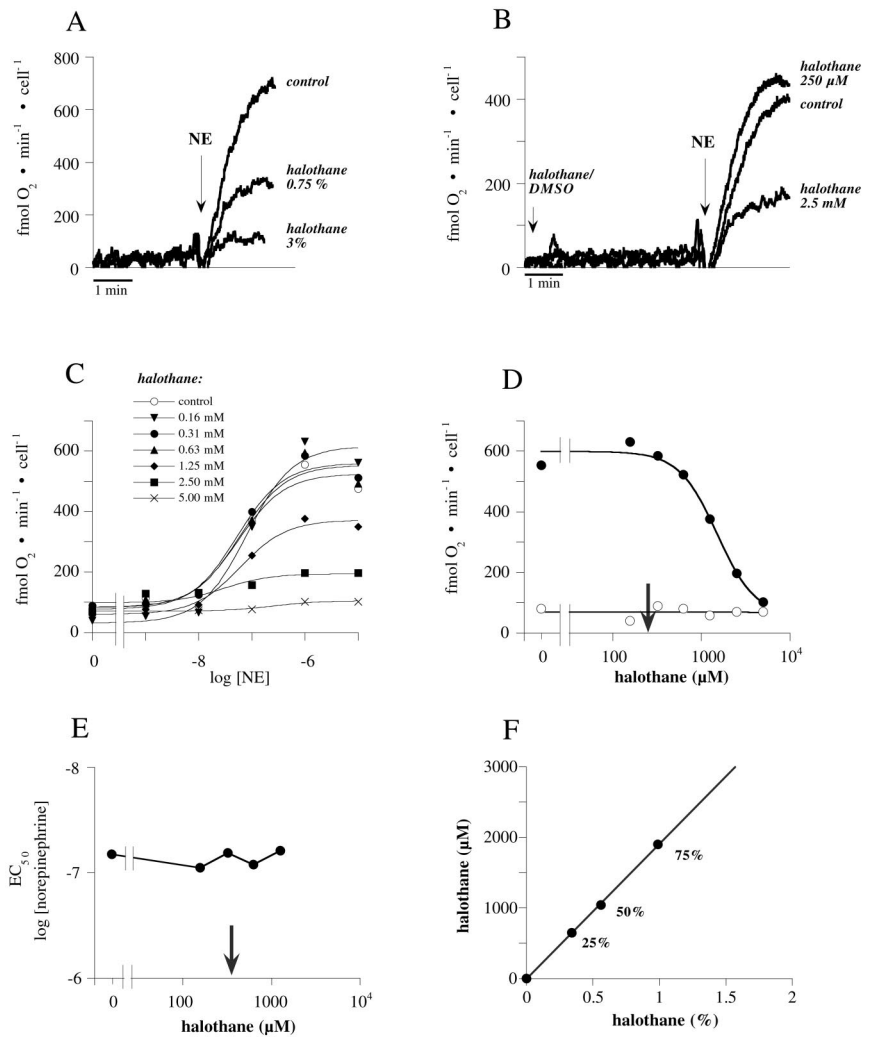

Fig. 1. The effect of halothane on oxygen consumption in isolated brown adipocytes. (*A*) Oxygen electrode traces showing the effect of halothane on the norepinephrine-induced rate of oxygen consumption in adipocytes. The cells were preincubated with halothane and were transferred to the oxygen electrode chamber. After 5-min incubation, 1 μM norepinephrine (final concentration) was added to the chamber. The curves are examples from one cell preparation. (*B*) Brown adipocytes were added to the oxygen electrode chamber and were incubated with the indicated nominal concentrations of halothane (in dimethyl sulfoxide [DMSO]) or DMSO alone (control) for 5 min before addition of 1 μM norepinephrine. The curves are running means from three different cell preparations. (*C*) The effect of different concentrations of halothane on the dose–response curves for norepinephrine. The experiments were performed as in (*B*), but with successive additions of norepinephrine to the indicated concentrations. All results are from the same cell preparation. Curves were drawn for simple Michaelis-Menten kinetics. (*D*) Dose–response curves for the effect of halothane on basal (open circles) and maximal norepinephrine-induced (closed circles) rates of oxygen consumption. The points represent values from (*C*). The curve for the maximal response was drawn for Michaelis-Menten kinetics with a Hill coefficient of 2, which yielded a better curve fit (principally as in Ohlson *et al.*[6]); IC_{50} for the nominal halothane concentration was thus calculated to 1.5 mM. The arrow indicates reported aqueous concentration of free halothane in buffer corresponding to the minimum alveolar concentration value (MAC_{aq}). (*E*) The effect of halothane on the EC_{50} values for norepinephrine. The values were calculated from (*C*) (see Materials and Methods). (*F*) Comparison between the inhibitory effect of halothane in gaseous form and the nominal concentration when added in DMSO. The curve was constructed from dose–response curves as that in (*D*), based on a compilation from a series of experiments (as that in *C*), with halothane added in solution, and a series of experiments (as that in *A*) with halothane added in gas form. From these, halothane concentrations yielding 25, 50, and 75% inhibition were read off and plotted.

bation tube. A total of 1.1 ml of the preincubated cell suspension was transferred to a oxygen electrode chamber for measurements of oxygen consumption.

Data Analysis

The data for the dose–response curves for norepinephrine were analyzed with a reiterative curve-fitting program (KaleidaGraph; Albeck Software) for best fit to the equation describing the rate of oxygen consumption as basal + V_{max} · ([norepinephrine]/([norepinephrine] + EC50)), where V_{max} = maximal increase of oxygen consumption. The same equation, but with fixed basal and V_{max} (from the 1-μM norepinephrine response in each trace), was used for calculation of EC_{50} for norepinephrine in figures 1E, 2D, 4C, 5, 6, 7C (10 μM norepinephrine for 390 mM ethanol). Other curve fits were performed as described in the figure legends.

Results

Volatile Anesthetics

Halothane. When administered by gaseous equilibration with the brown adipocytes in suspension, 3% halothane inhibited the maximal norepinephrine-induced rate of oxygen consumption by 70–80%[6] (fig. 1A); with 0.75% halothane (*i.e.*, 1 MAC), the inhibition was about 50% (fig. 1A). To confirm that halothane is also a potent inhibitor of thermogenesis when added directly to the cell suspension, the effect of halothane added in liquid form to the cells was examined. Various solvents for halothane were tested (water, ethanol, Intralipid® or DMSO). Of these, DMSO was found to be the most efficient solvent, based on the inhibitory effect of nominally equimolar halothane concentrations in the different solvents (not shown). When added as a solution in DMSO, halothane could inhibited oxygen consumption

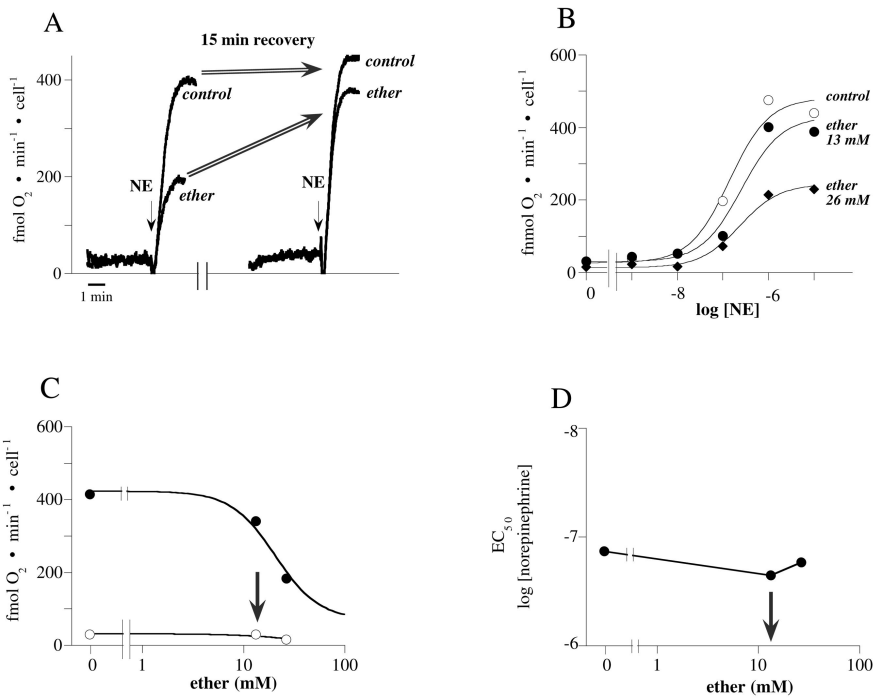

Fig. 2. The effect of ether on oxygen consumption in isolated brown adipocytes. (*A*) Recovery of the effect of ether on the rate of norepinephrine-induced oxygen consumption. One sample of the cell suspension was incubated with 26 mM ether (water in the controls) in the sealed oxygen electrode chamber for 5 min, before 1 μM norepinephrine was added. Another sample was incubated with ether for 5 min in the sealed chamber and was thereafter kept without the lid for 15 min. The chamber was then closed again and after 5 min, norepinephrine was added. Controls were treated similarly. The traces are the running means from two cell preparations, duplicate traces in each. (*B*) Dose–response curves for norepinephrine-induced rates of oxygen consumption with different concentrations of ether. The values are means from two different cell preparations. (*C*) Basal (open circles) and maximal norepinephrine-induced (closed circles) rates of oxygen consumption in different ether concentrations; values from (*B*). The curve for the maximal response was drawn as in fig. 1D. IC_{50} for ether was 20 mM. (*D*) EC_{50} values for norepinephrine, as an effect of different ether concentrations; values from (*B*). The arrows in (*C*) and (*D*) indicate MAC_{aq} for ether.

(fig. 1B) if added at sufficiently high doses. In figure 1C, dose–response curves for norepinephrine in the presence of different concentrations of halothane are shown. The results are compiled in figure 1D. There was no effect of halothane on the basal rates of oxygen consumption, but a dose-dependent inhibitory effect on the maximal norepinephrine-induced thermogenic response of the brown adipocytes was found; the IC_{50} for this inhibitory effect of halothane was nominally about 1 mM (1.1 ± 0.1 mM when results from nine experiments were pooled). The nominal concentration of halothane being equipotent to 0.75% gaseous halothane (1 MAC) was thus higher than the calculated free level corresponding to 1 MAC (0.25 mM),[10] which was expected because of binding of halothane to albumin[11] (see Material and Methods). In the absence of halothane, the EC_{50} value for norepinephrine was about 70 nM, in agreement with earlier observations.[6,26–28] Halothane had no effect on the EC_{50} value for norepinephrine stimulation of the cells (fig. 1E).

For a functional relation between the antithermogenesis effects of halothane administered in gaseous form and in DMSO, the results from a series of experiments, performed and analyzed as those described above, were compiled. From such dose–response curves, concentrations yielding a specified degree of inhibition were deduced, both when the addition was in gaseous or in liquid form; these corresponding degrees of inhibition are plotted in figure 1F. As seen, the effects of halothane added in gaseous form or in solution were parallel, with 1% halothane in gaseous form corresponding functionally to a nominal concentration of 2 mM halothane added in DMSO to the solution. Thus, also when administered in liquid form, the volatile anesthetic halothane had effects corresponding to those seen after gaseous administration.

Ether. Norepinephrine-induced oxygen consumption was reduced by about 50% when the brown adipocytes were exposed to 26 mM ether (2 MAC_{aq}). This inhibitory effect was reversible (fig. 2A) and dose-dependent (fig. 2B). The IC_{50} for the inhibition from the curve fit in figure 2C was calculated to be 20 mM. There was no effect of ether on norepinephrine sensitivity (fig. 2D).

Chloroform. The addition of 2.2 mM chloroform (which is twice the MAC_{aq}) led to an approximately 50% inhibition of the maximal rate of oxygen consumption, and the inhibition was reversible (fig. 3A), without effect on norepinephrine sensitivity (fig. 3B). (With higher concentrations of chloroform, the response to norepinephrine was totally abolished; data not shown.)

Tribromoethanol. Over a concentration range of 0.35–1.4 mM (0.1–0.4 mg/ml), tribromoethanol dose-dependently inhibited the norepinephrine-induced oxygen consumption; 2.8 mM totally abolished the response to norepinephrine (figs. 4A and 4B) without having any effect on the basal oxygen consumption. The IC_{50} for the inhibition was 0.6 mM. Tribromoethanol did not change the EC_{50} for norepinephrine (fig. 4C). Thus, tribromoethanol had an effect similar to that of the volatile anesthetic compounds.

Nonvolatile Anesthetics

The results of the examination of nonvolatile anesthetics are compiled in figure 5. It is evident that pentobarbital (fig.

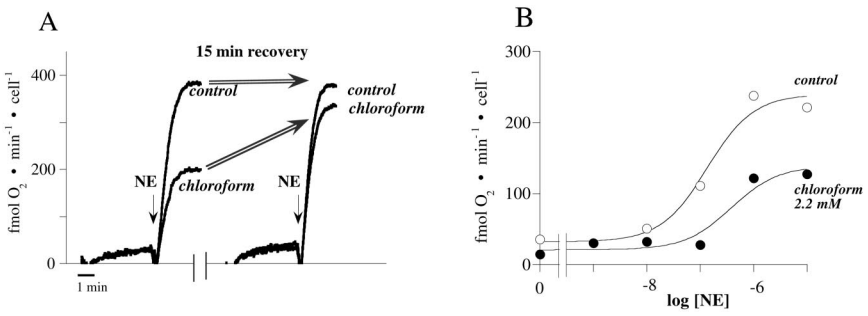

Fig. 3. The effect of chloroform on oxygen consumption in isolated brown adipocytes. (*A*) Recovery of the effect of chloroform on the rate of norepinephrine-induced oxygen consumption. Incubation of the cell suspension was performed as described for ether in fig. 2A but with 2.2 mM chloroform. The values are the running means from 2 different cell preparations, duplicate or triplicate traces in each. (*B*) Dose–response curves for norepinephrine-induced rates of oxygen consumption with 2.2 mM chloroform. The values are the means from triplicate traces in one cell preparation.

5A), propofol (fig. 5B), ketamine (fig. 5C), and urethane (fig. 5D) were all without effect on this experimental system, even when added at concentrations greatly exceeding relevant clinical concentrations. These anesthetics had no effect on basal oxygen consumption, on maximal norepinephrine-induced oxygen consumption, or on the sensitivity of the system to norepinephrine.

At concentrations of 25%, 50%, and 74%, nitrous oxide did not inhibit basal or maximal rates of oxygen consumption, and it had no effect on the EC_{50} for norepinephrine (fig. 6). (We earlier observed some inhibitory effect,[6] which could not be discerned in the current experiments.)

In concentrations as high as 390 mM (≈18‰), ethanol did not affect the basal or the maximal norepinephrine-induced rates of oxygen consumption in isolated adipocytes (figs. 7A and 7B). There was, however, a shift in the dose–response curve for norepinephrine: 390 mM ethanol shifted the EC_{50} for norepinephrine from about 90 nM in controls to about 450 nM in the ethanol-incubated cells (fig. 7C). Closer examination (Schild plot [not shown] and the analysis in figure 7C) revealed that ethanol acted as a competitive "pseudoantagonist" to norepinephrine with a K_B of 50 mM (pK_B, 1.3). The mechanism for this apparent competition is unknown.

Volatile Nonanesthetics (Nonimmobilizers)

To examine whether the inhibitory effect on thermogenesis of the volatile anesthetics was related to their anesthetic properties or simply an effect of their chemical characteristics, so-called volatile nonanesthetics were tested. The nonanesthetics 1,2-dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane, as well as halothane, were examined in parallel experiments (fig. 8A). As expected, halothane dose-dependently inhibited the norepinephrine-induced rate of oxygen consumption (fig. 8B; in agreement with fig. 1C). However, 1,2-dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane, even at 100 times the MAC_{pred} concentrations, did not affect the thermogenic capacity of the isolated brown adipocytes,

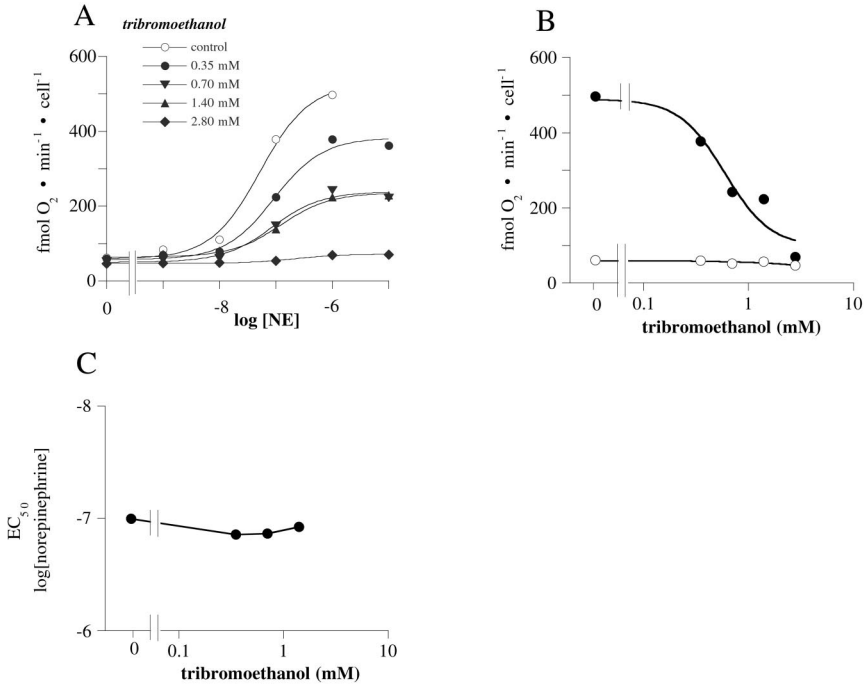

Fig. 4. The effect of tribromoethanol (Avertin) on oxygen consumption in isolated brown adipocytes. (*A*) Dose–response curves for norepinephrine-induced rates of oxygen consumption with different concentrations of tribromoethanol. (*B*) Basal (open circles) and maximal norepinephrine-induced (closed circles) rates of oxygen consumption in different tribromoethanol concentrations; values from (*A*). The curve for the maximal response was drawn to fit the equation used for halothane in fig. 1D. IC_{50} for tribromoethanol was calculated to be 0.6 mM. (*C*) EC_{50} for norepinephrine, as an effect of different tribromoethanol concentrations; values from (*A*). The values are the means from experiments in two different cell preparations.

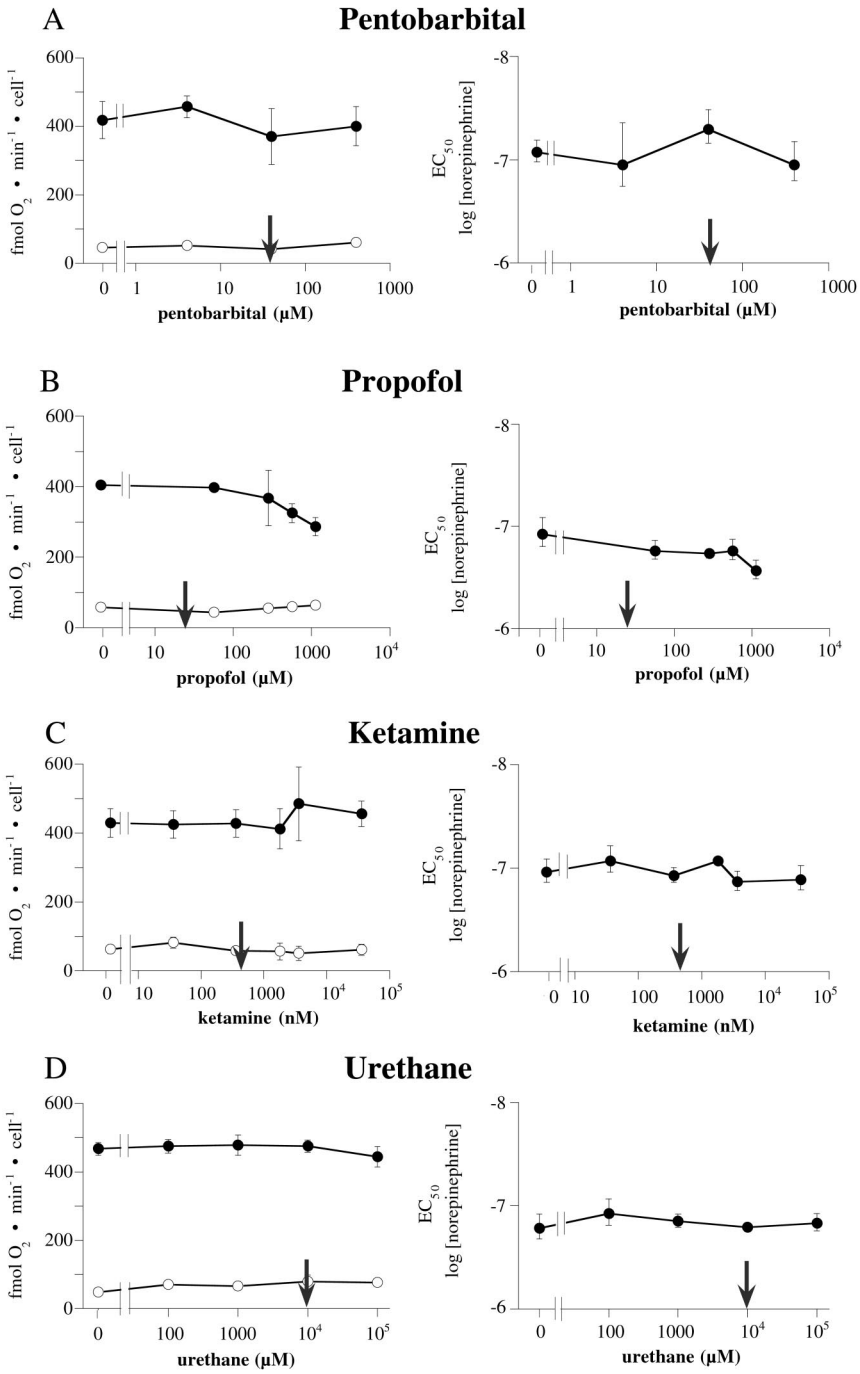

Fig. 5. The effect of nonvolatile anesthetics on oxygen consumption in isolated brown adipocytes. Experiments were performed principally as in figs. 1B and 1C and were analyzed as in figs. 1D and 1E. Brown adipocytes were incubated for 5 min with the indicated anesthetic drugs before successive additions of norepinephrine were made. (*Left*) Basal (open circles) and the maximal norepinephrine-induced (closed circles) rates of oxygen consumption. (*Right*) Corresponding EC_{50} values for norepinephrine. (*A*) Pentobarbital. The arrow indicates the free plasma concentration corresponding to a MAC (MAC_{aq}) value. (*B*) Propofol. The arrow indicates the plasma concentration measured during continuous infusion in anesthetized humans. (*C*) Ketamine. The arrow indicates the plasma concentration during anesthesia in humans. (*D*) Urethane. The arrow indicates the plasma concentration during anesthesia in rats. The results presented are means ± SE from two to three different cell preparations for each drug. Where not visible, error bars are smaller than the size of the symbol.

and the sensitivity to norepinephrine was not inhibited to any significant degree by either of the two compounds (figs. 8C–F).

Discussion

In the current investigation, we have identified a marked qualitative subdivision within anesthetic compounds, as revealed by studies in a model system: brown adipocytes. We demonstrate that norepinephrine-induced heat production in brown adipocytes is substantially inhibited by clinically relevant concentrations of a group of anesthetics consisting of volatile anesthetics, including tribromoethanol ("thermogenesis inhibitors"). Norepinephrine-induced heat production was, however, unaffected by anesthetic drugs not belonging to the group of volatile anesthetics, as well as by established volatile nonanesthetics ("thermogenesis noninhibitors"). The results are of interest for experimental studies in thermal physiology and may have implications for thermoregulation during anesthesia and surgery, especially in infants.

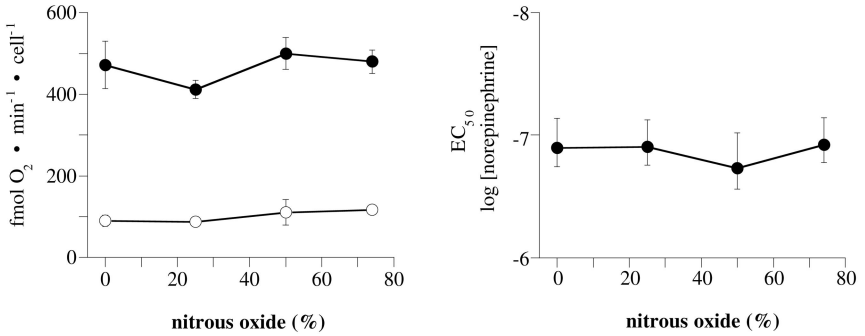

Fig. 6. The effect of nitrous oxide on oxygen consumption in isolated brown adipocytes. The experiments were performed as in fig. 1A but with the indicated nitrous oxide concentrations during the preincubation and with successive additions of norepinephrine (as in fig. 1C). The results are presented as in fig. 5 and are the means ± SE from at least three different cell preparations.

Nature of Thermogenesis Inhibitors and Noninhibitors

According to the outcome of this investigation, two groups of anesthetics can be identified: thermogenesis inhibitors and noninhibitors.

Thermogenesis inhibitors include the type-compound halothane (for which the phenomenon was originally observed),[6] as well as the clearly related compounds isoflurane and enflurane,[6] *i.e.*, volatile anesthetics. These compounds reversibly inhibit maximal norepinephrine-induced thermogenesis in brown adipocytes, without affecting the sensitivity to norepinephrine or the basal metabolism of the cells (see Ohlson *et al.*[6] and figs. 1, 8A, and 8B). Based on the current investigation, certain additional substances can be included (which may not obviously belong to the group of volatile anesthetics). One is tribromoethanol (fig. 4), which, although chemically rather similar to the volatile anesthetics, is administered by injection (and normally used for veterinary and experimental purposes only). Further, we can include the classic anesthetic agents diethyl ether and chloroform. It may have been anticipated that ether and chloroform would destroy and thus inhibit the thermogenic process in brown adipocytes in an nonspecific, membrane-dissolving fashion. However, the studies presented here (figs. 2 and 3) indicate that in the concentration range studied (*i.e.,* the clinically relevant range), the antithermogenic effect of ether and chloroform is reversible and specific, with characteristics similar to those of the volatile anesthetics used clinically today.

The thermogenesis noninhibitors (≈ nonvolatile anesthetics) include anesthetic substances that lack antithermogenic effect at clinically relevant concentrations, such as pentobarbital, propofol, ketamine, urethane, and nitrous oxide (figs. 5 and 6). Even at concentrations far exceeding the clinically relevant concentrations, these anesthetics are without effect on both maximal norepinephrine-induced thermogenesis and on norepinephrine

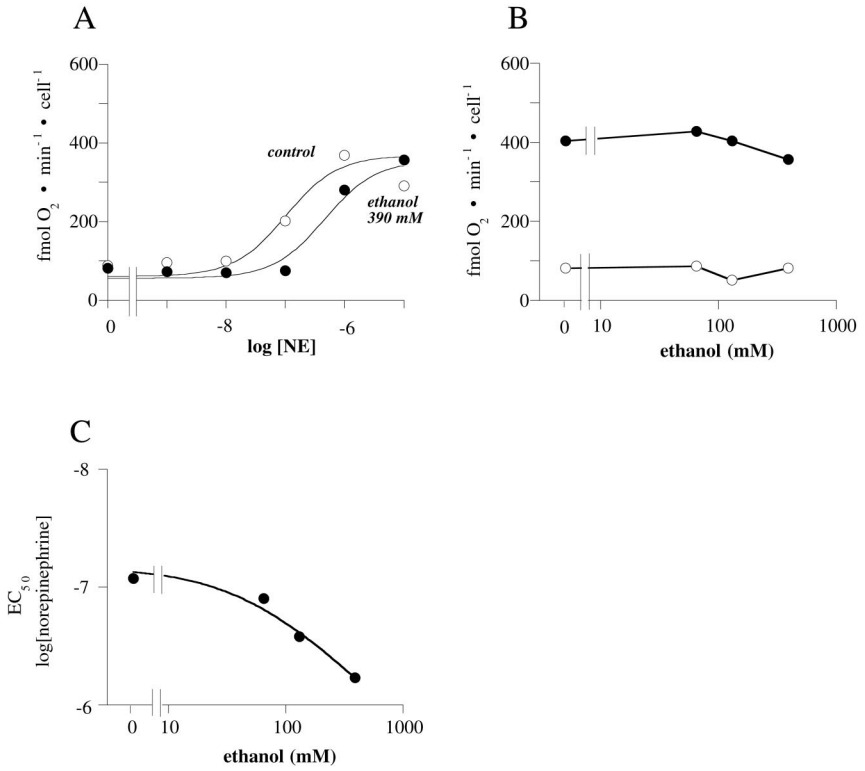

Fig. 7. The effect of ethanol on oxygen consumption in isolated brown adipocytes. (*A*) The effect of ethanol on the dose–response curve for norepinephrine-induced oxygen consumption. The experiments were performed as in fig. 1C, but with 390 mM ethanol added. Curves were drawn for simple Michaelis-Menten kinetics with defined V_{max} (the response to 1 μM norepinephrine for controls and to 10 μM for ethanol-treated cells). (*B*) Basal (open circles) and maximal norepinephrine-induced (closed circles) rates of oxygen consumption in different ethanol concentrations. (*C*) EC_{50} for norepinephrine, as an effect of different ethanol concentrations. The curve was drawn for best fit to the equation $IC_{50}(ethanol) = EC_{50} \cdot (1 + [ethanol]/K_B)$; K_B was thus calculated to be 50 mM ethanol. The values are the means from experiments in two different cell preparations.

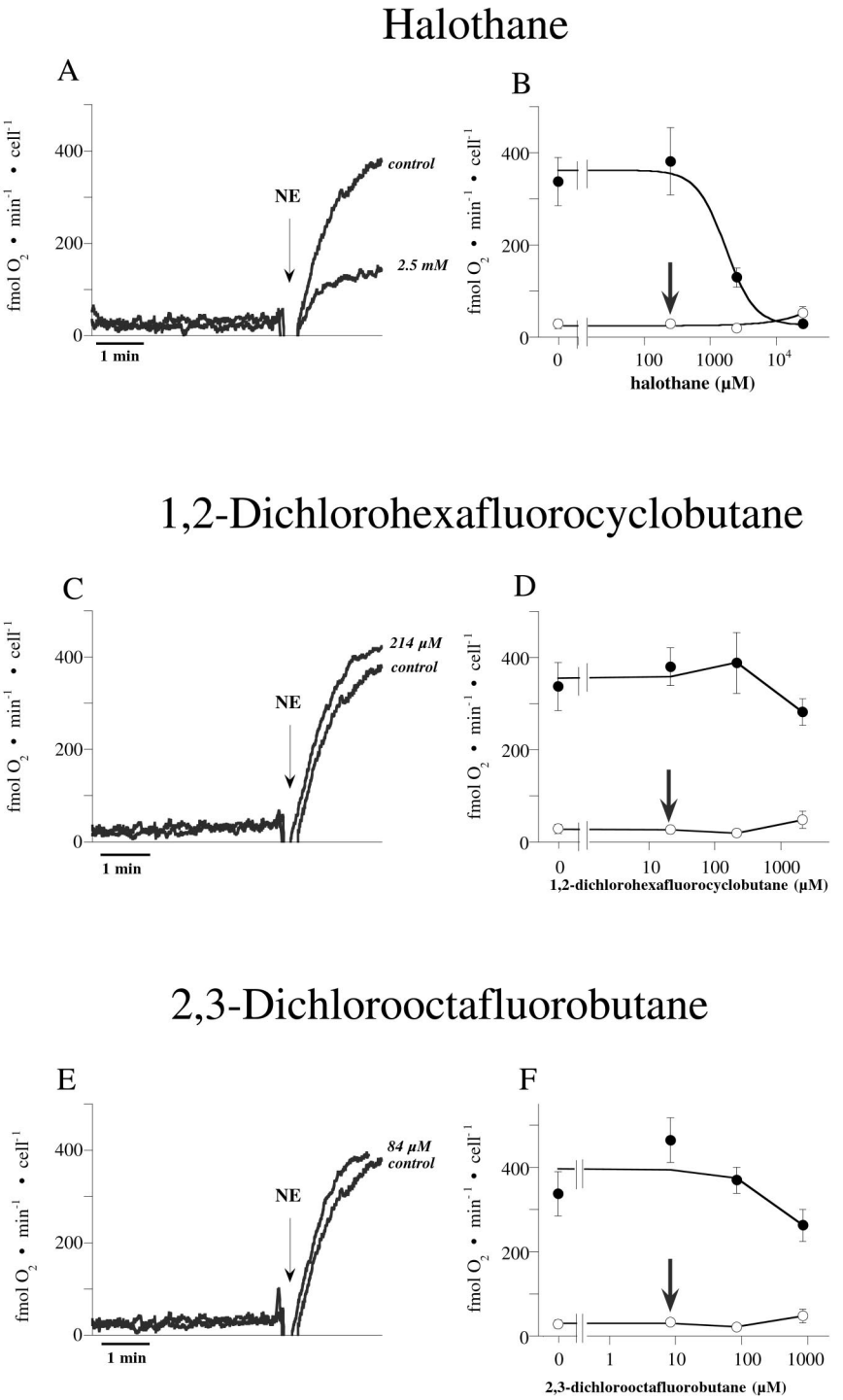

Fig. 8. The effect of (*A*) halothane, (*B*) 1,2-dichlorohexafluorocyclobutane, and (*C*) 2,3-dichlorooctafluorobutane on oxygen consumption in isolated brown adipocytes. The experiments were performed as in fig. 1B. The traces are the running means from four different cell preparations with 10-fold the predicted minimum alveolar concentration (MAC_{pred}). (*B*), (*D*), and (*F*) are values from experiments as in (*A*), (*C*), and (*E*), the means ± SE for 2-min basal rates of oxygen consumption (open circles) and for 1 min maximal response after 1 μM norepinephrine addition (closed circles). Values are from three to five different cell preparations. The curve in (*B*) was drawn as in fig. 1D. The arrow in (*A*) indicates the MAC_{aq} value and those in (*D*) and (*E*) the MAC_{pred} values.

sensitivity. This indirectly implies that the anesthetic effect of these substances does not involve receptors or processes that are part of the norepinephrine-induced pathways in brown adipocytes or of the function of the brown adipocytes in general. In agreement with this, it has been proposed for several of these substances[3,4,29] that they interact directly with pathways that are not present in brown adipocytes.

The group of thermogenesis noninhibitors may also be said to include ethanol. Ethanol has inhibitory effects on brown adipocytes (fig. 7), but it did not diminish the maximal norepinephrine-induced thermogenesis; instead, it had a competitive antagonist-like effect on the sensitivity to norepinephrine. This implies that the mode of action of ethanol is distinct from that of the thermogenesis inhibitors.

The noninhibitors also include the so-called nonanesthetics, which implies that the mode of action of the thermogenesis inhibitors is not simply related to them being halogenated carbon compounds.

Thermoregulation and Anesthesia

The question may be raised as to whether the functional subdivision of anesthetic compounds defined here experimentally as thermogenesis inhibitors and noninhibitors also reflects anesthetically relevant parameters, especially concerning thermoregulation during anesthesia.

If humans or animals during anesthesia are exposed to an environmental temperature below thermoneutral, the body temperature will decline.[30] This is observed both if anesthesia is induced by anesthetics of the thermogenesis inhibitory type (halothane[31] or isoflurane[32]) and if it is induced by the thermogenesis noninhibitors (urethane,[33] pentobarbital,[34,35] ketamine,[36] or nitrous oxide[37,38]). *A priori*, the decrease in body temperature could imply either that the peripheral thermogenic effectors are nonfunctional or that an alteration in central thermoregulatory control occurs. However, an experimental limitation of studies purportedly examining effects of anesthetics on the central control of body temperature is that the parameters followed are in reality peripheral responses to body cooling (or heating), *i.e.,* the initiation of thermoregulatory effector responses such as vasoconstriction, shivering thermogenesis, and nonshivering thermogenesis. The studies of central control are thus performed under the implicit assumption that the compound under study does not affect the effector mechanism directly. From the observations presented here, it is clear that if one of the thermogenesis inhibitory anesthetics is used, this prerequisite is not fulfilled concerning nonshivering thermogenesis.

The reason for the decrease in body temperature during anesthesia is not that anesthesia is associated with a loss of thermoregulation, but rather that a broadening of the tolerated core temperatures occurs. Thus, a lowering of the threshold for vasoconstriction is observed for the volatile anesthetics (thermogenesis inhibitors such as halothane,[31] isoflurane,[39] or enflurane[40]), but a similar lowering is also observed for propofol, a thermogenesis noninhibitor.[41] Similarly, a lowering of the threshold for shivering is observed for volatile anesthetics (thermogenesis inhibitors) in both humans (isoflurane[39]) and animals (isoflurane[42]), but a similar lowering is also observed for the thermogenesis noninhibitor propofol.[41] Thus, the broadening of the span of tolerated core temperatures is an effect of unconsciousness or anesthesia as such, rather than a direct effect of the anesthetic agent.

It is the implication of the current experiments that the outcome of the examination of whether the central control of nonshivering thermogenesis is also functional during anesthesia must depend on the type of anesthetic used.

In experimental animals, the capacity for nonshivering thermogenesis is generally estimated as the increase in the metabolic rate following norepinephrine injection; this procedure results in a stimulation of the brown adipocytes *in situ*. Experiments of this type are currently routinely performed in conscious animals but were earlier often performed in anesthetized animals. However, serendipitously, in most thermoregulatory studies performed during anesthesia, the thermogenesis noninhibitors urethane or pentobarbital were used. There are several reports that a thermogenic response is elicited by norepinephrine during anesthesia with such thermogenesis noninhibitors, *e.g.*, in barbiturate-anesthetized animals,[43–47] whereas the thermogenic effect of norepinephrine is fully eliminated when animals are anesthetized by the thermogenesis inhibitor halothane.[7]

When a cold-defense response is elicited physiologically, *i.e.,* by cold exposure, nonshivering thermogenesis is also observable in experimental animals anesthetized with thermogenesis noninhibitors, *e.g.*, barbiturates[48,49] and urethane[50,51] (or if they are treated with the nonanesthetic 1,2-dichlorohexafluorocyclobutane[52]). In contrast, cold-induced nonshivering thermogenesis is inhibited by halothane[53] and isoflurane.[52] There are, however, experimental indications showing that the central control of nonshivering thermogenesis is not eliminated during anesthesia, even when thermogenesis inhibitors are used: there is still cold-induced nervous stimulation of the tissue during isoflurane anesthesia,[54,55] but according to the results presented here and earlier,[6] such stimulation cannot result in thermogenesis in these animals.

It has been concluded that there is an absence of nonshivering thermogenesis in adult humans during anesthesia.[56,57] However, as the anesthetic used was the thermogenesis inhibitor isoflurane, it is inherently impossible from such experiments to conclude whether the absence is the result of a central effect (inhibition of the center controlling nonshivering thermogenesis) or a peripheral effect (the direct inhibitory effect of volatile anesthetics on nonshivering thermogenesis)—but it must be added that it is generally doubted that normal adults can exhibit nonshivering thermogenesis.

There are also implications that cold stress during halothane anesthesia does not increase metabolism in infants,[58] who—in contrast to adults—should possess functional brown adipose tissue and thus cold-induced nonshivering thermogenesis. There is one study in which activation of nonshivering thermogenesis has been investigated in infants anesthetized with thermogenesis noninhibitors.[59] The anesthetics used were propofol (which, according to fig. 5, is a thermogenesis noninhibitor) and fentanyl, which is probably also a thermogenesis noninhibitor. (Fentanyl as such was not examined here; however, as much as 50 μg/ml morphine does not affect norepinephrine-induced oxygen consumption in brown adipocytes [our unpublished data]). The propofol–fentanyl-anesthetized neonates vasoconstricted when the esophageal temperature reached 36°C, but no increase in the rate of oxygen

consumption was noticed, not even when the core temperature was as low as 34°C. This seems to indicate that nonshivering thermogenesis was not activated during anesthesia, but it is remarkable that humans possessing brown adipose tissue (*i.e.,* neonates and infants) would behave differently from other mammals in this respect, and further studies may be necessary to clarify if a species difference exists.

Because of their high amounts of brown adipose tissue and thus their high potential for nonshivering thermogenesis, neonates and infants should otherwise theoretically be in a better situation during anesthesia than adults, as they could produce more heat to compensate heat loss and thus keep a higher body temperature, even when a myorelaxant is administered (although they also lose heat faster than adults because of their high surface/volume ratio). The results from the current study imply that heat balance problems may be aggravated during anesthesia with volatile anesthetics.

Could the brown-adipocyte model be helpful for elucidating the molecular basis for anesthesia?

It may be discussed whether the brown adipocyte system is an "irrelevant" or a "relevant" model system[60] for analysis of the mechanism of anesthesia. Of course, thermogenesis is in itself not directly involved in the state of anesthesia, but as long as the mediation of anesthesia is not known, it is difficult to distinguish between "relevant" and "irrelevant" model systems. Indeed, the effect of the anesthetics on the brown adipocytes does represent an inhibition of a specific cellular pathway, which in these cells leads to thermogenesis but which in another cell system may be the one controlling the degree of consciousness.

The brown adipocyte model system has therefore the potential to allow for identification of a possible novel molecular mechanism, common for the volatile anesthetic–induced inhibition of thermogenesis in these cells and for the volatile anesthetic–induced abolishment of consciousness in target cells in the central nervous system.

In summary, it was found in an experimental *in vitro* model of nonshivering thermogenesis (norepinephrine-induced oxygen consumption in brown adipocytes) that volatile anesthetic agents inhibit thermogenesis, whereas nonvolatile anesthetics agents do not. In addition, so-called nonanesthetics (nonimmobilizers), although chemically similar to volatile anesthetic agents, do not inhibit thermogenesis. These results are of interest from an experimental thermoregulatory perspective and have direct implications on temperature balance during anesthesia and surgery, especially in neonates and infants. The results may also be of interest for the understanding of anesthetic mechanisms at a molecular level.

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