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Role of adenosine in the ethanol-induced potentiation of the effects of general anesthetics in rats

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Abstract

Acetate, derived from ethanol metabolism in the liver, is released into the circulation and utilized in many tissues including the brain. The subsequent metabolism of acetate results in the production of adenosine that has a number of effects in the central nervous system. The purpose of the present studies, therefore, was to investigate the contribution of metabolically generated adenosine to the ethanol-induced potentiation of the inhalational agents isoflurane and sevoflurane. Changes in the anesthetic requirement for isoflurane and sevoflurane were determined in rats using the tail-clamp procedure. Both ethanol and sodium acetate reduced anesthetic requirement for isoflurane and sevoflurane in a dose-dependent fashion. The effect of acetate on anesthetic requirement was completely blocked by the administration of the adenosine receptor blocker, 8-phenyltheophylline. The ethanol-induced reduction in anesthetic requirement, however, was only partially blocked by 8-phenyltheophylline. Direct intracerebroventricular (i.c.v.) administration of the water-soluble adenosine receptor blocker, 8-sulfophenyltheophylline, also completely blocked the effect of acetate and partially blocked the effect of ethanol. This i.c.v. administration demonstrates that the actions of ethanol and acetate on anesthetic requirement are a central nervous system effect. The i.c.v. administration of the adenosine A_1 receptor subtype agonist, *R*-phenylisopropyl adenosine, potentiated the anesthetic effects of isoflurane and suggests that the A_1 receptor mediates the observed potentiation of anesthetic effect. This is further supported by the concomitant administration of 5-*N*-ethylcarboxamido adenosine, a non-selective adenosine agonist, with the selective A_1 antagonist, 8-cyclopentyltheophylline, showing A_1 receptor potentiation of anesthetic requirements. The studies show that (1) acetate potentiates the anesthetic effects of the inhalational anesthetics, sevoflurane and isoflurane; (2) acetate contributes in part to the effect of ethanol on anesthetic potency through metabolically generated adenosine; (3) these effects are likely mediated via adenosine A_1 receptor subtypes. © 1997 Elsevier Science B.V.

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1. Introduction

The minimal alveolar concentration has proven to be a reliable and remarkably constant measurement of anesthetic potency in animals and in man (Quasha et al., 1980). The acute administration of several central nervous system depressants including ethanol is known to potentiate the effects of inhalational general anesthetics. Indeed a dose-related reduction in the minimal alveolar concentration of halothane and isoflurane has been demonstrated following the administration of ethanol to rodents (Johnstone et al., 1975; Parikh, 1976). The mechanism of this interaction is, however, unknown.

Following ethanol ingestion, about 90% is metabolized in the liver to acetaldehyde and acetate. Approximately 70% of the acetate generated is released from the liver and is metabolized in extrahepatic tissues (Lundquist et al., 1962), including the brain (Busch, 1953; Van den Berg et al., 1966; Berl and Frigyesi, 1969). The metabolism of acetate requires adenosine triphosphate and coenzyme A and results in the production of acetyl coenzyme A, adenosine monophosphate and pyrophosphate. Adenosine monophosphate is further metabolized by the enzyme 5-nucleotidase to produce adenosine (Kiviluoma et al., 1989; Lianey and Lowenstein, 1978; Puig and Fox, 1984).

Adenosine, a naturally occurring purine nucleoside, has

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powerful effects in the central nervous system. Many of these effects such as sedation, sleep, and motor incoordination, are similar to those of ethanol (Dunwiddie et al., 1984; Dar et al., 1983; Clark and Dar, 1989). Also adenosine has been found to reduce the anesthetic requirement for halothane in dogs (Seitz et al., 1990). We have recently shown that some of the central effects of ethanol result from the metabolism of acetate to generate adenosine and that this acetate-adenosine-mediated portion of the ethanol effect can be attenuated by the adenosine receptor antagonist, 8-phenyltheophylline (Carmichael et al., 1991). We therefore postulate that following ethanol administration, the metabolically derived acetate, acting via adenosine, would directly augment anesthetic potency. The purpose of the present study is to investigate the role of the acetate-adenosine system in the ethanol-induced reduction in general anesthetic requirement.

2. Material and methods

With approval of the University of Toronto Animal Care Committee, male Sprague-Dawley rats (Charles River Breeding Laboratories, St. Constance, Quebec, Canada) weighing 250–350 g were studied. The rats were housed in the animal facility in the Medical Sciences Building of the University of Toronto. They were maintained in a constant temperature and humidity environment with a 12 h light/dark cycle. Prior to study, the rats were fasted overnight with water ad libitum.

2.1. Determination of anesthetic requirement

Minimal alveolar concentration was determined as previously described (Carmichael et al., 1991; White et al., 1974; Crawford et al., 1992). In brief, spontaneously breathing rats were placed in clear Plexiglas chambers and were exposed to the anesthetic agent in 100% oxygen at a flow rate of 5 l/min. The inspired anesthetic concentration was continuously monitored using a calibrated infrared medigas analyzer (Sensor Medics LB2, Anaheim, CA, USA). Following administration of the anesthetic for 20 min, the rats were stimulated using a rubber-clad 6 inch hemostat clamped on the mid-portion of the tail to the first ratchet position. Gross, purposeful movement of the head, extremities, or body was considered a positive response. If a positive response was observed, the inspired concentration was increased by 10% and the stimulus reapplied after 20 min. If a negative response was initially observed, the inspired concentration was decreased by 10% and the stimulus reapplied after 20 min. This was repeated until a negative response was followed by a positive response. Minimal alveolar concentration was defined as the inspired concentration of anesthetic midway between the highest concentration with a positive response and the lowest concentration with a negative response.

2.2. Implantation of intracerebroventricular (i.c.v.) cannulas

Under pentobarbital anesthesia (65 mg/kg), the rats were placed in a stereotactic frame. The i.c.v. cannula in a plastic bolt (model 0-80, Plastics One, Roanoke, VA, USA) was positioned 0.08 cm posterior and 0.20 cm lateral to the bregma at a depth of 0.35 cm (Swanson, 1992). The i.c.v. cannula penetrated to a depth of 4.5–5.0 mm and was held in place using cranioplastic methylmethacrylate cement (Plastics One). The rats were given an intramuscular injection of penicillin G (15 mg) and were allowed to recover for 5–6 days prior to study. The i.c.v. injections of 10 μl were administered through a special 28 gauge internal cannula using a micro syringe (Hamilton, Reno, NV, USA) over a period of 20 s.

2.3. Drug administration

Ethanol was diluted in normal saline to a 10% solution and was administered by intraperitoneal (i.p.) injection in doses of 0.5, 1.0, and 1.5 g/kg. Control rats received an equal volume (1 ml/100 g body weight) of normal saline, i.p. Sodium acetate was prepared as a 10% solution (pH 7.4) and was administered by i.p. injection in doses of 0.5, 1.0, and 1.5 g/kg. Control rats received an equivalent volume of saline, i.p. The adenosine receptor blocker, 8-phenyltheophylline (Sigma, St. Louis, MO, USA) that is approximately 7-fold more potent than theophylline and lacks significant phosphodiesterase activity (Daly, 1985), was dissolved in 0.1 M saline at pH 11.4 and administered by i.p. injection at a dose of 12 mg/kg in a volume of 0.3 ml. Control rats received an equal volume of 0.1 M saline adjusted to pH 11.4. The water-soluble adenosine antagonist, 8-sulfophenyltheophylline (Research Biochemicals International, Natick, MA, USA) (Daly et al., 1985), with a potency similar to theophylline, was dissolved in saline at pH 7.4 and was administered by direct injection into the cerebral ventricles (i.c.v. injection) at a dose of 250 μg in a volume of 10 μl, prior to placing rats into the chambers. Control rats received an equivalent volume of saline adjusted to pH 7.4 by i.c.v. injection. The 106-fold selective adenosine A_1 receptor agonist, *R*(−)*N*6-(2-phenylisopropyl) adenosine (Bruns et al., 1986) (Research Biochemicals International) was administered by i.c.v. injection at a dose of 0.05–0.1 μg at pH 7.4 in a volume of 10 μl. The A_1-A_2 adenosine receptor agonist 5-*N*-ethyl-carboxamido adenosine (Research Biochemicals International) was administered by i.c.v. injection at a dose of 0.2–0.4 μg at pH 7.4 in a volume of 10 μl. The 132-fold selective adenosine A_1 receptor antagonist 8-cyclopentyltheophylline (Bruns et al., 1986) (Research Biochemicals International) was administered by i.c.v. injection at a dose of 0.6 μg at pH 7.4 in a volume of 10 μl. Control rats received an i.c.v. injection of an equal volume of normal saline adjusted to pH 7.4.

Following ethanol administration, the increase in circulating acetate reaches a plateau of approximately 1 mM within 5–10 min (Carmichael et al., 1991; Giles et al., 1986; Nuutinen et al., 1985). It was previously reported that in the rat the administration of 1 g/kg of sodium acetate resulted in circulating concentrations of acetate similar to those observed following doses of ethanol above 0.5 g/kg (Carmichael et al., 1991).

The inhalational anesthetic agents, isoflurane (1-chloro-2,2,2-trifluoroethyl-difluoromethyl ether) (Ohmeda, Rexdale, Ontario, Canada) and sevoflurane (fluoromethyl-2,2,2-trifluoro-*l*-[trifluoromethyl] ethyl ether) (Maruishi Pharmaceuticals, Osaka, Japan), were administered in 100% oxygen using thermocompensated vaporizers (Ohmeda, Rexdale, Ontario, Canada). A comparison was made between isoflurane and the newer inhalational anesthetic, sevoflurane, which is known to differ in physicochemical and anesthetic properties (Frink et al., 1992; Eger, 1994).

2.4. Experimental design

To determine the effect of the administration of ethanol and of acetate on anesthetic requirement, rats were randomly injected with increasing doses of either agent prior to placement into the breathing chambers and determining anesthetic potency. To determine the role of adenosine receptors in the ethanol- and acetate-induced decrease in anesthetic requirement, 8-phenyltheophylline was injected 10 min prior to the administration of ethanol or acetate. The rats were then placed into the chambers and sevoflurane or isoflurane was administered in 100% oxygen for 20 min before application of the test stimulus. This 20 min period allowed for the equilibration of inspired, expired and tissue concentrations (White et al., 1974).

To specifically identify the involvement of brain adenosine receptors in anesthetic requirement, the rats were given 8-sulfophenyltheophylline by i.c.v. injection prior to the administration of ethanol or sodium acetate. They were immediately placed into the chambers and isoflurane was administered in 100% oxygen for 20 min before application of the test stimulus. To determine the adenosine receptor subtype involved in the potentiation of isoflurane anesthesia, rats were administered: (a) the A_1-A_2 agonist, 5-*N*-ethylcarboxamido adenosine, with or without the A_1-specific antagonist, 8-cyclopentyltheophylline, or (b) the A_1-specific agonist, *R*-phenylisopropyl adenosine. These drugs were administered by i.c.v. injection prior to placement of the animals into the chambers and the determination of anesthetic requirement.

Data are presented as mean values ± S.E.M. The data were analyzed using a one-way and two-way analysis of variance (ANOVA) (SAS Programming, Cary, NC, USA). Post-hoc analysis of the differences between mean values were assessed using Duncan's multiple range test.

3. Results

The effect of acetate and ethanol with and without 8-phenyltheophylline on the minimal alveolar concentration of sevoflurane is shown in Table 1. The minimal alveolar concentration value of sevoflurane was 2.20 ± 0.03% inspired concentration. The administration of ethanol resulted in a significant change ($P < 0.001$) in anesthetic requirement, reducing the minimal alveolar concentration of sevoflurane by 53% at 1.5 g/kg ethanol. Likewise, the administration of sodium acetate resulted in a significant ($P < 0.001$) dose-dependent reduction in the minimal alveolar concentration of sevoflurane by 10% at 0.5 g/kg and by 26% at 1.5 g/kg sodium acetate. The minimal alveolar concentration of sevoflurane was not significantly altered by administration of the adenosine receptor blocker, 8-phenyltheophylline. The administration of 8-phenyltheophylline significantly antagonized the ethanol-induced reduction in the minimal alveolar concentration of sevoflurane ($P < 0.001$). The acetate-induced reduction in minimal alveolar concentration of sevoflurane was completely blocked by 8-phenyltheophylline. The overall effect of 8-phenyltheophylline on the acetate-induced reduction in

Table 1
Effect of acetate and ethanol with and without 8-phenyltheophylline on the minimal alveolar concentration (MAC) of sevoflurane

	MAC of sevoflurane	MAC of sevoflurane + 8-phenyltheophylline (12 mg/kg)	P [a]
Saline (1 ml/kg, i.p.)	2.20 ± 0.03 [b] (10)	2.09 ± 0.03 (6)	N.S.
Acetate (0.5 g/kg, i.p.)	1.98 ± 0.02 [c] (6)	2.20 ± 0.02 (8)	< 0.001
Acetate (1.0 g/kg, i.p.)	1.79 ± 0.02 [d] (7)	2.06 ± 0.01 (8)	< 0.001
Acetate (1.5 g/kg, i.p.)	1.63 ± 0.02 [d] (7)	2.13 ± 0.04 (8)	< 0.001
Ethanol (1.5 g/kg, i.p.)	1.02 ± 0.03 [d] (9)	1.27 ± 0.03 (9)	< 0.001

[a] MAC of sevoflurane with vs. without 8-phenyltheophylline.
[b] Values shown are means ± S.E.M. Numbers in parentheses are number of animals.
[c] $P < 0.01$ vs. saline controls.
[d] $P < 0.001$ vs. saline controls.

Table 2
Effect of acetate and ethanol with and without 8-phenyltheophylline on minimal alveolar concentration (MAC) of isoflurane

	MAC of isoflurane	MAC of isoflurane + 8-phenyltheophylline (12 mg/kg)	P [a]
Saline (1 ml/kg, i.p.)	1.40 ± 0.03 [b] (12)	1.33 ± 0.02 (8)	N.S.
Acetate (0.5 g/kg, i.p.)	1.28 ± 0.02 [c] (8)	1.35 ± 0.01 (7)	< 0.001
Acetate (1.0 g/kg, i.p.)	1.23 ± 0.01 [c] (8)	1.35 ± 0.02 (7)	< 0.001
Acetate (1.5 g/kg, i.p.)	1.13 ± 0.03 [c] (8)	1.26 ± 0.01 (12)	< 0.001
Ethanol (0.5 g/kg, i.p.)	1.19 ± 0.02 [c] (10)	1.27 ± 0.01 (8)	< 0.01
Ethanol (1.0 g/kg, i.p.)	1.06 ± 0.02 [c] (13)	1.09 ± 0.02 (8)	N.S.
Ethanol (1.5 g/kg, i.p.)	0.90 ± 0.01 [c] (8)	1.00 ± 0.02 (8)	< 0.001

[a] MAC of isoflurane with vs. without 8-phenyltheophylline.
[b] Values shown are means ± S.E.M. Numbers in parentheses are number of animals.
[c] $P < 0.001$ vs. saline controls.

minimal alveolar concentration was significant ($P < 0.0001$) when compared to sodium acetate alone.

The effect of acetate and ethanol with and without 8-phenyltheophylline on minimal alveolar concentration of isoflurane is shown in Table 2. The minimal alveolar concentration value of isoflurane was 1.40 + 0.03% inspired concentration. The administration of ethanol resulted in a significant ($P < 0.001$) and dose-dependent reduction in anesthetic requirement, reducing the minimal alveolar concentration of isoflurane by 15% at 0.5 g/kg and 36% at 1.5 g/kg ethanol. The administration of sodium acetate resulted in a significant ($P < 0.001$) dose-dependent reduction in minimal alveolar concentration of isoflurane by 8% at 0.5 g/kg and by 19% at 1.5 g/kg sodium acetate. The minimal alveolar concentration of isoflurane was not significantly altered by the administration of 8-phenyltheophylline (12 mg/kg). The overall effect of 8-phenyltheophylline on the ethanol-induced reduction in the minimal alveolar concentration of isoflurane was significant ($P < 0.025$), resulting in a shift of the dose-response curve to the right. The slopes of these two lines were not different. The acetate-induced reduction in the minimal alveolar concentration of isoflurane was completely blocked by 8-phenyltheophylline. The overall reduction by 8-phenyltheophylline of the acetate-induced reduction in minimal alveolar concentration was significant ($P < 0.002$), when compared to sodium acetate alone.

The effect of ethanol and acetate with and without 8-sulfophenyltheophylline on minimal alveolar concentration of isoflurane is shown in Table 3. The i.c.v. administration of the water-soluble adenosine receptor blocker, 8-sulfophenyltheophylline, had no effect on the minimal alveolar concentration value of isoflurane. This dose of 8-sulfophenyltheophylline, however, completely blocked the minimal alveolar concentration reducing effects of i.p. administered sodium acetate while only partially reducing the effect of ethanol on the minimal alveolar concentration of isoflurane. At the 0.5 g/kg dose of ethanol, 8-sulfophenyltheophylline reversed 38% of the minimal alveolar concentration reducing effect, whereas at 1.5 g/kg, 22% of the ethanol effect was antagonized.

The effect of adenosine analogues, *R*-phenylisopropyl

Table 3
Effect of ethanol and acetate with and without 8-sulfophenyltheophylline on minimal alveolar (MAC) concentration of isoflurane

	MAC of isoflurane	MAC of isoflurane (+ 8-sulfophenyltheophylline, 250 μg)
Saline (10 μl)	1.40 ± 0.01 (12)	1.39 ± 0.01 (6)
Ethanol (0.5 g/kg i.p.)	1.19 ± 0.02 [a] (10)	1.27 ± 0.01 [a,b] (6)
Ethanol (1.0 g/kg i.p.)	1.06 ± 0.02 [a] (13)	1.17 ± 0.02 [a,b] (6)
Ethanol (1.5 g/kg i.p.)	0.90 ± 0.01 [a] (8)	1.01 ± 0.01 [a,b] (6)
Acetate (1.5 g/kg i.p.)	1.13 ± 0.02 [a] (8)	1.38 ± 0.02 [b] (6)

Ethanol and acetate were given by intraperitoneal injection prior to the administration of isoflurane. 8-Sulfophenyltheophylline was given by intracerebroventricular injection prior to the administration of ethanol or sodium acetate. Values shown are means + S.E.M. Numbers in parentheses are number of animals.
[a] $P < 0.001$ vs. saline control.
[b] $P < 0.025$ vs. without 8-sulfophenyltheophylline.

Table 4
Effect of adenosine analogues, *R*-PIA (*R*-phenylisopropyl adenosine) and NECA (5-*N*-ethylcarboxamido adenosine), on MAC (minimal alveolar concentration) for isoflurane

	MAC of isoflurane	MAC of isoflurane (+ 8-cyclopentyltheophylline)
Saline (10 μl)	1.40 ± 0.01 (7)	1.37 ± 0.01 (6)
R-PIA (0.05 μg)	1.19 ± 0.01 [a] (6)	–
R-PIA (0.10 μg)	0.90 ± 0.03 [a] (7)	–
NECA (0.2 μg)	1.17 ± 0.01 [a] (6)	1.40 ± 0.01 [b] (6)
NECA (0.4 μg)	1.01 ± 0.02 [a] (6)	1.38 ± 0.02 [b] (7)

Saline, 8-cyclopentyltheophylline, *R*-PIA, and NECA were given by intracerebroventricular injection prior to the administration of isoflurane. Values shown are means ± S.E.M. Numbers in parentheses are number of animals.
[a] $P < 0.0001$ vs. saline control.
[b] $P < 0.001$ vs. without 8-cyclopentyltheophylline.

adenosine and 5-*N*-ethylcarboxamido adenosine, on minimal alveolar concentration of isoflurane is shown in Table 4. The i.c.v. administration of the 106-fold selective adenosine A_1 receptor agonist, *R*-phenylisopropyl adenosine, produced a dose-dependent reduction in the minimal alveolar concentration of isoflurane ($P < 0.001$). Similarly, the i.c.v. administration of the non-selective A_1-A_2 adenosine receptor agonist, 5-*N*-ethylcarboxamido adenosine, also resulted in a dose-dependent reduction in minimal alveolar concentration of isoflurane ($P < 0.001$). However, the i.c.v. pretreatment with the 132-fold selective adenosine A_1 receptor antagonist, 8-cyclopentyltheophylline, completely attenuated the effect of 5-*N*-ethylcarboxamido adenosine on the minimal alveolar concentration of isoflurane.

4. Discussion

A number of centrally acting drugs are known to alter anesthetic requirement. Several narcotic analgesics decrease the minimal alveolar concentration of inhalational anesthetics (Munson et al., 1965; Saidman and Eger, 1964; Hoffman and DiFazio, 1970). Barbiturates, benzodiazepines, and nitrous oxide reduce minimal alveolar concentration in a dose-related fashion (Taylor et al., 1957; Perisho et al., 1971; Tsunoda et al., 1973). Clonidine, the prototypical α_2-adrenoceptor agonist, has also been shown to reduce anesthetic requirement in both animals (Bloor and Flacke, 1982; Maze and Tranquilli, 1991) and in humans (Ghignone et al., 1988; Kaukinen and Pyykko, 1979; Engelman et al., 1989). This is also the case with the calcium channel blocker, nimodipine (Schwartz et al., 1991).

The acute ingestion of ethanol is also known to decrease the anesthetic requirement of inhalational anesthetics. In rats, for example, the intraperitoneal injection of ethanol reduced the minimal alveolar concentration of halothane in a dose-related fashion by 10% and 60% following a dose of 0.8 g/kg and 4.0 g/kg respectively (Parikh, 1976). Similarly, ethanol induced a dose-dependent reduction in the minimal alveolar concentration of isoflurane in mice (Johnstone et al., 1975). The present findings of an ethanol-induced, dose-dependent reduction in the anesthetic requirement for sevoflurane and isoflurane are in agreement with these earlier reports.

The mechanism by which ethanol modifies the central effects of anesthetics is not known. Earlier studies have generally assumed that the effects of ethanol on minimal alveolar concentration are the result of the lipophilic or hydrophobic membrane effects of the ethanol molecule per se. There is now considerable evidence suggesting that these direct effects of ethanol are mediated through ligand-gated ion channels such as γ-aminobutyric acid, *N*-methyl-D-aspartate, etc. (Miller et al., 1987; Lovinger et al., 1989; Harris, 1990; Tanelian et al., 1993). We have previously postulated that the effect of ethanol on the central nervous system can be separated into two components. The first component, the hydrophobic effect, is dose-related and depends on the concentration of ethanol acting directly on cell membrane components. The second, non-dose-related effect is dependent on ethanol metabolism and is maximal at low doses of ethanol due to saturation of the alcohol dehydrogenase system (Carmichael et al., 1991). Since the latter metabolic component is due to adenosine production during acetate metabolism (Lianey and Lowenstein, 1978; Puig and Fox, 1984; Orrego and Carmichael, 1991), these two effects of ethanol can be differentiated by blocking adenosine receptors with methylxanthines such as 8-phenyltheophylline. We have previously shown in rats that the motor incoordinating effects of ethanol are partially blocked by 8-phenyltheophylline. This supports the notion that part of the central nervous system effects of ethanol on motor coordination are the result of a metabolism-dependent, acetate-adenosine-mediated mechanism (Carmichael et al., 1991).

It is conceivable that the potentiation of an anesthetic by ethanol is a simple additive, direct membrane effect of two anesthetic agents in the ascending part of the dose-response curve. If this were true, the administration of 8-phenyltheophylline would not be expected to attenuate the additive combination of ethanol and anesthetic. This, however, was not the case. The administration of 8-phenyltheophylline, that by itself had no effect on minimal alveolar concentration, reduced the potency of the combined inhalational agent and ethanol demonstrating an adenosine-mediated component.

According to our hypothesis, only the metabolism-dependent effects of ethanol, i.e. those related to the acetate-adenosine system, should be blocked by 8-phenyltheophylline administration. Moreover, this inhibition should be greater at low doses of ethanol where a greater proportion of the effects of ethanol are metabolism-dependent. This assumption has been confirmed in experiments with ethanol and acetate on motor coordination (Carmichael et al., 1991). Our hypothesis also predicts that the effect of acetate should be completely blocked by 8-phenyltheophylline. Our results have confirmed this prediction. Therefore, as with other effects of ethanol, the acetate-adenosine mechanism operates only where the metabolism of acetate into acetyl coenzyme A occurs (Lianey and Lowenstein, 1978; Puig and Fox, 1984).

The effect of ethanol on the splanchnic circulation to increase portal blood flow represents a model where the response is entirely mediated by the acetate-adenosine system (Orrego et al., 1988; Carmichael et al., 1988). In the central nervous system the role of the acetate-adenosine system is only partially responsible for the actions of ethanol on anesthetic potency. It is clear that a number of other effects of alcohol may not depend on ethanol metabolism and accordingly they should not be affected by 8-phenyltheophylline. An alternate mechanism by which

ethanol could affect central nervous system function through an adenosine-mediated effect has been proposed (Nagy et al., 1990; Nagy, 1992). These authors have demonstrated the presence of an ethanol-sensitive adenosine transporter in cells. An inhibition of this transporter in the presence of ethanol would lead to an increase in extracellular adenosine and could thereby modulate some of the effects of ethanol. This effect would occur at higher doses of ethanol, would be independent of the effects of acetate, and would be blocked by adenosine receptor antagonists.

To further demonstrate that the effects of ethanol and acetate were mediated by central nervous system adenosine receptors, the water-soluble adenosine receptor blocker 8-sulfophenyltheophylline was administered directly into the cerebrospinal fluid by i.c.v. injection prior to the administration of ethanol or acetate. This adenosine receptor blocker by itself had no effect on minimal alveolar concentration, similar to 8-phenyltheophylline. These studies produced virtually identical results to those with the peripheral administration of 8-phenyltheophylline. This demonstrates that the observed effects of ethanol and of acetate on minimal alveolar concentration are mediated by adenosine receptors in the central nervous system.

Acetate is rapidly taken up into the brain by a carrier-mediated process (Oldendorf, 1973) and metabolized into acetyl coenzyme A (Berl and Frigyesi, 1969). An effect of acetate on the central nervous system, however, has only recently been recognized. Indeed, acetate has usually been thought of as a biologically inert intermediate of ethanol metabolism (Lands, 1991). We have shown recently, however, that acetate produces motor incoordination in rats and decreases spontaneous motor activity in mice (Carmichael et al., 1991). The significant dose-dependent reduction in minimal alveolar concentration of sevoflurane and isoflurane following the administration of acetate provides a further demonstration that this molecule does indeed have profound effects in the central nervous system.

Two adenosine receptor subtypes, A_1 and A_2, have been identified and characterized in brain tissue (Daly, 1985; Londos et al., 1980). To determine which receptor subtype mediated the observed reductions in minimal alveolar concentration, the 106-fold selective adenosine A_1 receptor agonist *R*-phenylisopropyl adenosine and the 132-fold selective A_1 antagonist 8-cyclopentyltheophylline (Bruns et al., 1986) in combination with a potent A_1-A_2 agonist, 5-*N*-ethylcarboxamido adenosine, were used. Both *R*-phenylisopropyl adenosine and 5-*N*-ethylcarboxamido adenosine produced a dose-dependent reduction in the minimal alveolar concentration of isoflurane. When the injection of 5-*N*-ethylcarboxamido adenosine was combined with the i.c.v. administration of 8-cyclopentyltheophylline, no reduction in the minimal alveolar concentration of isoflurane was observed. These results using receptor ligands that were more than 100-fold selective suggest that it is the adenosine A_1 receptor subtype that mediates the minimal alveolar concentration reducing effects in the brain. These findings are similar to those of Dunwiddie et al. (1984) and Dar (1990) showing that other central nervous system effects of adenosine are mediated by the A_1 receptor subtype. This contrasts to the vasodilatory effects of adenosine that are mediated by the A_2 receptor subtype (Carmichael et al., 1988; King et al., 1990). Hemodynamic studies following the intracerebral administration of both *R*-phenylisopropyl adenosine and 5-*N*-ethylcarboxamido adenosine have shown that these adenosine receptor agonists produce a reduction in blood pressure (Barraco et al., 1986). It is important to note, however, that the reductions in blood pressure that would occur at doses used in the present study would not be sufficient to affect the determination of anesthetic potency (Cullen, 1986).

In summary, the present data suggest that the direct hydrophobic or lipophilic effects of ethanol, probably on ligand-gated ion channels, play a predominant role in the potentiation of minimal alveolar concentration of inhalational anesthetics. The complete reversal of the effect of acetate on minimal alveolar concentration by 8-phenyltheophylline and 8-sulfophenyltheophylline suggests that the effect of acetate is entirely mediated by central nervous system adenosine receptors. The ethanol-induced reduction in minimal alveolar concentration, on the other hand, was partially inhibited by 8-phenyltheophylline and 8-sulfophenyltheophylline, showing that only part of the ethanol effect on anesthetic requirement is due to the metabolism-dependent effects of ethanol and the generation of adenosine. Further, the use of the selective adenosine A_1 receptor agonists and antagonists and A_2 agonist suggests that this effect may be mediated by the adenosine A_1 receptor subtype.

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