Differential effects of volatile and intravenous anesthetics on the activity of human TASK-1

C. Putzke,1 P. J. Hanley,2 G. Schlichthörler,3 R. Preisig-Müller,3 S. Rinné,3 M. Anetseder,4 R. Eckenhoff,5 C. Berkowitz,1 T. Vassiliou,1 H. Wulf,1 and L. Eberhart1

1Department of Anesthesiology and Critical Care Medicine, Philipps-University Marburg, Germany; and 2Institute für Physiologie II, Universitätsgymnasion Münster; 3Institute of Physiology, Philipps-University Marburg; 4Klinik und Poliklinik für Anästhesiologie, Universitätsgymnasion Würzburg; and 5Department of Anesthesiology and Critical Care, University of Pennsylvania Health System, Philadelphia, Pennsylvania

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Volatile anesthetics have been shown to activate various two-pore (2P) domain K+ (K2P) channels such as TASK-1 and TREK-1 (TWIK-related acid-sensitive K+ channel), and mice deficient in these channels are resistant to halothane-induced anesthesia. Here, we investigated whether K2P channels were also potentially important targets of intravenous anesthetics. Whole cell patch-clamp techniques were used to determine the effects of the commonly used intravenous anesthetics etomidate and propofol on the acid-sensitive K+ current in rat ventricular myocytes (which strongly express TASK-1) and selected human K2P channels expressed in Xenopus laevis oocytes. In myocytes, etomidate decreased both inward rectifier K+ (I_K1) and acid-sensitive outward K+ current at positive potentials, suggesting that this drug may inhibit TASK channels. Indeed, in addition to inhibiting guinea pig Kir2.1 expressed in oocytes, etomidate inhibited human TASK-1 (and TASK-3) in a concentration-dependent fashion. Propofol had no effect on human TASK-1 (or TASK-3) expressed in oocytes. Moreover, we showed that, similar to the known effect of halothane, sevoflurane and the purified R-(-)- and S- (+)-enantiomers of isoflurane, without stereoselectivity, activated human TASK-1. We conclude that intravenous and volatile anesthetics have dissimilar effects on K2P channels. Human TASK-1 (and TASK-3) are insensitive to propofol but are inhibited by supraclinal concentrations of etomidate. In contrast, stimulatory effects of sevoflurane and enantiomeric isoflurane on human TASK-1 can be observed at clinically relevant concentrations.

et al. (24) reported that TASK-1−/− mice are less sensitive (indexed by tail-withdrawal reflexes) to halothane and isoflurane. These gene knockout studies, as well as the observation that volatile anesthetics activate TASK-like currents in various neuronal preparations (32, 33, 35), implicate a contributory role for K2P channels in the mechanism of anesthesia in vivo.

General anesthesia is usually initiated by injection of intravenous anesthetics such as propofol (2,6-diisopropylphenol) or, where better hemodynamic stability is called upon, etomidate (2-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate). Unlike the case for volatile anesthetics, little attention has been given to the possible effects of these drugs on K2P channel activity. In this study, we aimed to determine whether activation of TASK channels was common to volatile and intravenous anesthetics. We compared the effects of volatile and intravenous anesthetics on acid-sensitive K+ currents in isolated rat ventricular myocytes and oocytes expressing selected K2P channels. Putzke et al. (31) recently reported, in accord with others (26), that the rat heart expresses TASK-1, TASK-3, and TREK-1, but real-time PCR analyses indicated that purified ventricular myocytes predominantly express TASK-1. Moreover, Putzke et al. (31) found that the novel TASK-1 blocker A293 increased the duration of the action potential in rat ventricular myocytes by about 30%. Thus TASK-1 is probably an important modulator of excitability in both the central nervous system and the heart.

METHODS AND MATERIALS

The experimental procedures were approved by the Regierungspräsidium (Giessen, Germany), and the study was performed according to the Helsinki convention for the use and care for animals.

Isolation of ventricular myocytes. Ventricular myocytes were isolated from collagenase-perfused rat hearts as essentially described elsewhere (9). Myocytes were seeded onto 3-mm diameter cell culture dishes (Nunc, Denmark). After a dish was mounted on the stage of an inverted microscope (Olympus, Japan) equipped with a CCD camera, a U-shaped perfusion chamber was seated on the floor of the dish, and cells were superfused with solution containing (in mM) 140 NaCl, 5.4 KCl, 1 CaCl2, 1 MgCl2, 0.33 NaH2PO4, 10 glucose, and 5 HEPES (pH 7.4 with NaOH).

Patch-clamp experiments with myocytes. Electrophysiological recordings were performed using the whole cell patch-clamp recording configuration under voltage-clamp conditions. Patch pipettes were pulled from borosilicate glass capillaries (Science Products, 10.220.32.247) on 21 June 2017.
Hofheim, Germany) with a two-stage puller (DMZ Universal Puller, Zeitz-Instrumente, Munich, Germany), and the resistance was 4–8 MΩ. Membrane currents were measured using an Axo-patch 200B amplifier (Axon Instruments, Burlingame, CA), and data were analyzed using custom software (LabView, National Instruments, Austin, TX). Slow voltage ramps (6 mV/s) were applied between −60 mV and +30 mV. The pipette solution contained (in mM) 60 KCl, 65 potassium-glutamate, 5 EGTA, 2 MgCl₂, 3 K₂ATP, 0.2 Na₂GTP, and 5 HEPES (pH 7.2 with KOH). To avoid the confounding effect of specific ion channels, the bath solutions were modified by addition of 1 mM propranolol (nonselective β-receptor blocker), 3 μM nisoldipine (L-type Ca²⁺ channel blocker), and 2 μM glibenclamide (ATP-sensitive K⁺ channel blocker). In preliminary experiments, we found that outward current at +30 mV was reduced by 19 ± 7% (n = 4) in the presence of these combined blockers. Na⁺ currents were minimized by applying a slow voltage-step protocol.

Channel expression in Xenopus oocytes. Oocytes were harvested from Xenopus laevis as previously described (22, 25) and injected with cRNA for human TASK-1 (hTASK-1, 1.5 ng), hTASK-3 (0.05 ng), or gpKir2.1 (2.5 ng). After 48 h incubation, oocytes were placed in a Perspex recording chamber and superfused with solution containing (in mM) 96 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, 2.5 Na-pyruvate, and 5 HEPES (pH 7.4 with NaOH). Whole cell K⁺ currents were measured by two-electrode voltage clamp. Pipette solutions contained 3 M KCl. All myocyte and oocyte experiments were performed at room temperature. In contrast to the myocyte experiments, all oocyte experiments were performed in the absence of ion channel blockers.

Fig. 1. Stimulatory effect of halothane on 2-pore K⁺ domain (K₂P) channels. A: representative recording showing the effect of halothane on human TASK-1 (hTASK-1) expressed in oocytes. Current was elicited by applying voltage ramps between −120 mV and +20 mV. B: summary of data obtained using oocytes (n = 3 in each case) expressing hTASK-1 or rat TASK-1 (TASK-1). C: representative current-voltage relations of ventricular myocytes before (control) and after application of 1 mM halothane. Whole cell current was measured during voltage ramps applied between −60 mV and +30 mV; summary of data obtained using n = 8 myocytes (D).

Fig. 2. Sevoflurane stimulates hTASK-1. A: typical recording showing the effect of 1 mM sevoflurane and acidic pH on hTASK-1 current in an oocyte. Sequential voltage ramps were applied between −120 mV and +20 mV, and current measured at 0 mV was plotted as a function of time. B: sevoflurane (1 mM) and acidic pH (pH 5.5) were applied as indicated by the solid bars. Summary of data was obtained by using n = 4 oocytes.
Drugs. Methanandamide was obtained from Calbiochem (Germany), and etomidate and propofol were acquired from Braun (Melsungen, Germany). Sevoflurane and racemic isoflurane were obtained from Abbott (Wiesbaden, Germany), and pure enantiomers of isoflurane were prepared by chiral chromatography. All other chemicals were purchased from Sigma (Germany) or Fluka (Germany). Methanandamide was added directly to solutions, whereas stock solutions of etomidate and propofol were prepared in dimethylsulfoxide. The volatile anesthetics used in this study [halothane, racemic isoflurane, R(-)-isoflurane, S(+)-isoflurane, and sevoflurane] were dissolved directly into the solution, which was held in a specially designed syringe (AnaConDa, Hudson RCI, Upplands Väsby, Sweden) impermeable to these agents. Using HPLC and gas chromatography, we confirmed that there was no loss of anesthetic content between the syringe and the recording chamber. We tested volatile anesthetics at a concentration of 1 mM, which approximately corresponds to three times the MAC value for each agent (10), where 1 MAC is defined as the minimal alveolar concentration required to prevent movement in response to a painful stimulus in 50% of animals. Thus volatile anesthetics were tested at the upper end of clinically relevant concentrations.

Statistical analyses. All values are reported as means ± SE with P < 0.05 as a criterion for significance. Data were evaluated using SigmaPlot (Aspire Software International, Leesburg, VA) and an ANOVA was used to test for significant differences were necessary.

RESULTS

Effects of halothane on whole cell currents and Kir2p channel activity. To confirm that halothane activates TASK-1, we challenged oocytes expressing hTASK or rat TASK-1 (rTASK-1) with this halogenated hydrocarbon (Fig. 1, A and B). As shown in Fig. 1A, halothane (1 mM) increased hTASK-1 current whereas, characteristic of TASK-1, acidic pH (pH 5.5) inhibited the current. Halothane similarly activated rTASK-1 expressed in oocytes, as summarized in Fig. 1B. The 30–40% increase in current at +20 mV (Fig. 1B) is in line with the observations of Patel et al. (30), who reported that 1 mM halothane increased hTASK-1 (expressed in COS cells) by ~50%.

Since TASK-1 is strongly expressed in rat ventricular myocytes (31), we tested whether halothane increased outward K+ current in isolated myocytes at positive potentials (Fig. 1, C and D). Figure 1C shows representative current-voltage relations measured in a ventricular myocyte before (control) and after application of 1 mM halothane. Peak steady-state outward current in the −60 to 0 mV range of the voltage-clamp ramp protocol occurred between −50 and −40 mV. This current is mediated by members of the Kir2.x subfamily of inwardly rectifying K+ (Kir) channels (3, 4, 27, 29), and it was essentially unaffected by halothane. However, at more positive potentials, halothane exerted a stimulatory effect on the outward current (Fig. 1C). On average, 1 mM halothane increased outward current at +30 mV [a potential at which Kir2.x channels are inactive (31)] by 24 ± 3% (n = 8) (Fig. 1D). At acidic pH, 1 mM halothane increased outward current at positive potentials by only 12 ± 2% (n = 8; Fig. 1D). Taken together, the above data confirm that halothane activates acid-sensitive hTASK-1 and rTASK-1 and indicate that a major component of halothane-induced outward current in native rat myocytes is mediated by TASK-1.

Effects of sevoflurane and isoflurane R(-)- and S(+)-enantiomers on human TASK-1. Given the potential importance of TASK-1 in the mechanism of volatile anesthetic action (30), we investigated whether the halogenated ether sevoflurane activated hTASK-1. Moreover, S(+)-isoflurane has been reported to increase human TRESK (TWIK-related spinal cord K+ channel) current significantly more than the R(-)-enan-
Etomidate inhibits acid-sensitive outward \( K^+ \) currents at positive potentials in myocytes. We predicted that etomidate would inhibit the TASK-1 contribution to outward current at positive potentials in myocytes. Consistent with TASK inhibition, we found that etomidate inhibited whole cell current generated at positive potentials, as shown in Fig. 5A. Etomidate additionally inhibited inwardly rectifying \( K^+ \) currents (\( I_{K1} \)), but we initially focused on currents at more positive potentials. At the highest concentration tested, etomidate decreased the current at +30 mV by ~20% (Fig. 5B). To assess the contribution TASK-1 normally makes to the outward current in myocytes, we employed the TASK-1-selective blocker methanandamide, an analogue of the endogenous cannabinoid anandamide (20). When 10 \( \mu M \) methanandamide was introduced to myocytes, the outward current was decreased from 252 ± 19 to 194 ± 20 pA (\( n = 18 \)), a 25% reduction (Fig. 5, C and D). In complementary experiments, we varied extracellular pH because TASK-1 and TASK-3 are strongly inhibited by extracellular acidosis (6, 16, 17, 21). As shown in Fig. 5, E and F, a decrease of the extracellular pH from 7.4 to 6.0 inhibited the outward current in ventricular myocytes. On average, acidification of the bath solution decreased current from 252 ± 22 to 189 ± 15 pA (\( n = 25 \)) at +30 mV, a decrease of ~25%. In contrast, alkalinization increased the current from 306 ± 46 to 332 ± 43 pA (\( n = 10 \)), an 11% increase. Etomidate (1 mM) did not inhibit residual outward current measured at pH 5.0 (Fig. 5F). These data suggest that etomidate inhibits the TASK components TASK-1 > TASK-3 (31) of the outward current in ventricular myocytes.

Etomidate inhibits TASK-1 and TASK-3 expressed in oocytes. To test whether activation of TASK was common to volatile and intravenous anesthetics, we investigated the effects of the intravenous anesthetic etomidate on hTASK-1 and hTASK-3 expressed in oocytes. Typical recordings of whole cell current elicited by voltage ramps (−80 to +40 mV) are shown in Fig. 4A. The component mediated by TASK-1 was inhibited by superfusing the oocyte with acidic solution (pH 5.5). Etomidate did not activate but, instead, inhibited this current in a dose-dependent manner (Fig. 4B; \( n = 5 \)). Human TASK-3 was also inhibited by etomidate (Fig. 4, C and D; \( n = 3 \)) to a similar extent. These data demonstrate collectively that etomidate has opposite effects on TASK current compared with volatile anesthetics.

**Fig. 4.** Etomidate inhibits hTASK-1 and TASK-3 expressed in oocytes. A: representative recordings showing the inhibitory effects of etomidate and acidic pH on TASK-1 currents. Current-voltage relations were performed 2 min after solution changes. B: concentration-response relation (obtained using \( n = 5 \) oocytes) of etomidate on acid-sensitive (hTASK-1) current measured at 0 mV (IC\(_{50}\) value, 119 \( \mu M \)). C and D: inhibitory effects of etomidate and pH on hTASK-3 current measured at 0 mV (IC\(_{50}\) value, 128 \( \mu M \)). Data obtained using \( n = 3 \) oocytes.
Inhibitory action of etomidate on inwardly rectifying $K^+$ current. In the next series of experiments, we explored the inhibitory effect of etomidate on $I_{K1}$. In Fig. 6A, the effect of various concentrations of etomidate on ramp-generated current (-60 to 0 mV) in a representative experiment is shown, and concentration-response data are summarized in Fig. 6B. At the highest concentration tested (1 mM), average peak current was decreased from 209 ± 14 to 67 ± 7 pA ($n = 11$). This current is primarily mediated by Kir2.x channels (3, 29), but to confirm that etomidate could inhibit channels of this family, we examined the effect of this anesthetic on current in oocytes expressing guinea pig (gp) Kir2.1. Indeed, etomidate inhibited Ba$^{2+}$-sensitive Kir2.1 current in a dose-dependent fashion, as shown in Fig. 6C. The dose-response data from four experiments are summarized in Fig. 6D.

$hTASK-1$ and $hTASK-3$ are insensitive to the intravenous anesthetic propofol. We next tested whether propofol also inhibited TASK. In contrast to etomidate, propofol had no
significant effect on hTASK-1 (Fig. 7, A and B; n = 3) or hTASK-3 (Fig. 7, C and D; n = 3) expressed in oocytes. However, in whole cell recordings performed with ventricular myocytes, propofol (40 μM) increased outward currents at positive potentials and it appeared to inhibit \( I_{K1} \) (Fig. 8), and we speculated that propofol activates a chloride channel. Consistent with a Cl\(^-\) conductance under our experimental conditions, the difference (control vs. propofol treatment) current had a reversal potential of about \(-20\) mV (Fig. 8). These data show that propofol has no effect on TASK but, in myocytes, propofol may stimulate an unidentified chloride channel.

**DISCUSSION**

**Activation of TASK-1 by volatile anesthetics.** Activation of the K\(_{CP}\) channels TASK-1 and TREK-1 at clinically relevant concentrations has been postulated to play a role in the mechanism of action of volatile anesthetics (12, 24, 30), at least in rodents. Here we showed that sevoflurane, racemic isoflurane and its purified \( R-(-) \) and \( S-(+)- \) enantiomers activated hTASK-1 expressed in oocytes. In addition, we showed that halothane similarly increased hTASK-1 and rTASK-1 expressed in oocytes, and, furthermore, the stimulatory action of halothane on outward current (at positive potentials) in rat ventricular myocytes was sensitive to acidic pH. TASK-1 is strongly expressed in the rodent heart (8, 13, 26, 31) and in the brain. Among the various molecular targets of volatile anesthetics (10), activation of TASK-1 may have important effects in both of these organs. TASK-1 and TASK-3 are expressed in the thalamus (14), and Meuth et al. (28) deduced that these channels mediate halothane-induced outward current in thalamocortical neurons, recorded in rat thalamic slices, because the current could be blocked by extracellular acidification and bupivacaine, a nonspecific TASK blocker (18). However, mice lacking TASK-1 are only weakly resistant to anesthesia induced by halothane (24), suggesting that TASK-1 is not critical for the in vivo action of volatile anesthetics. TASK-1 knockout mice may have been functionally compensated by expression of TASK-3 or other gene products, and generation of TASK-1/TASK-3 double knockout mice may help to better assess the relative importance of TASK in anesthesia.

In the rodent heart, TASK-1 is expressed in ventricular myocytes (13, 31) and richly present in the conduction system (8). We found that about 20% of the outward current in rat ventricular myocytes was acid sensitive at positive potentials, and the strong inhibitory effect of the blocker methanandamide suggested that TASK-1 is the dominant subtype expressed in rat myocytes (19, 31). Recently, Besana and colleagues (1) nicely demonstrated that TASK-1 current could be inhibited in isolated mouse ventricular myocytes by platelet-activating factor, culminating in action potential repolarization abnormalities. In further work, Putzke et al. (31) demonstrated that inhibition of TASK-1 with the novel blocker A293 prolonged the action potential of rat myocytes, indicating that TASK-1 current contributes to normal repolarization. Interestingly, it has been known...
for a long time that volatile anesthetics shorten the cardiac action potential (10). This effect could be explained, at least in part, by TASK-1 activation.

**TASK-1 and I_{K1} is inhibited by the intravenous anesthetic etomidate but insensitive to propofol.** Our data suggest that “activation” of TASK-1 or TASK-3 is not essential for the mechanism of anesthesia induced by etomidate and propofol. Etomidate, in fact, inhibited hTASK-1 and hTASK-3 expressed in oocytes. Furthermore, consistent with TASK-1 (and possibly TASK-3) inhibition, etomidate inhibited pH-sensitive currents at positive potentials in ventricular myocytes. In addition, we observed that etomidate inhibited I_{K1} in myocytes. Homomeric Kir2.1 and Kir2.2 have been deduced to be the major channels underlying I_{K1} in mammalian hearts (36, 37) and, consistent with our myocyte data, we found that etomidate inhibited gplKir2.1 expressed in oocytes. In accord with our observations, Buljubasic et al. (2) reported that 60 μM etomidate weakly inhibited I_{K1} measured in isolated canine ventricular myocytes. However, etomidate reaches peak concentrations of about 10 μM after intravenous injection (5, 7) and, thus, significant inhibition of TASK-1 or TASK-3 would not be expected following administration of clinically relevant doses. Moreover, hTASK-1 and hTASK-3 were essentially unresponsive to the structurally distinct intravenous anesthetic propofol, even at concentrations exceeding predicted peak plasma concentrations (~40 μM) achieved in a clinical setting (11).

In conclusion, the major novel findings of this study are that volatile and intravenous anesthetics do not share the ability to activate human TASK-1. High concentrations of etomidate, but not propofol, inhibit TASK-1 (and TASK-3), which makes a modest contribution to outward current at positive potentials in the heart. Etomidate additionally inhibits Kir2.x channels. In contrast to the intravenous anesthetics, we found that, in addition to halothane, sevoflurane, and the purified R-(−)- and S-(+)-enantiomers of isoflurane stimulate hTASK-1 activity at clinically relevant concentrations. We speculate that stimulation of TASK-1 current by volatile anesthetics contributes to
the cerebral depressive and cardiac action potential shortening actions of these drugs.

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