Effect of $K^+$ Channel Blockade with Tetraethylammonium on Anesthetic-induced Relaxation in Canine Cerebral and Coronary Arteries


The mechanism by which volatile anesthetics produce their direct effects on vascular smooth muscle remains unknown. The authors previously reported that volatile anesthetics decrease both Ca$^{2+}$ and $K^+$ currents, however the role of Ca$^{2+}$-activated $K^+$ channels during the vasorelaxation by anesthetics has not been investigated. The purpose of this study was to determine whether blockade of the $K^+$ channel alters the response to volatile anesthetics. Responses were studied in canine middle cerebral arteries and proximal and distal canine coronary arteries. Vascular rings (2-mm length) were suspended in tissue baths, and isometric tension was recorded. Rings were constricted with 40 mM KCl and prostaglandin F$_2$α (middle cerebral arteries only) and subsequently exposed to enflurane (3.25%), halothane (1.35%), and isoflurane (2.1%). Volatile anesthetics produced vasorelaxation with relative potency in order: enflurane > halothane > isoflurane. The procedure was repeated in the presence of the K$^+$ channel blocker tetraethylammonium chloride (TEA, 20 mM). In all groups of vessels TEA alone elicited either no increase or only a transient increase in tension, however constrictions to both agonists were augmented in the presence of TEA. The presence of TEA significantly augmented anesthetic-induced vasorelaxation in small and large coronary vessels and in middle cerebral arteries. However, this effect was more pronounced in the cerebral as compared to coronary arteries. Constrictions produced in cerebral vessels by 15 μM prostaglandin F$_2$α were comparable with constrictions produced by 5 μM prostaglandin F$_2$α in the presence of TEA. The subsequent relaxant response of these vessels to enflurane was also comparable in the two groups. This finding suggests that the augmented relaxant response to enflurane in the presence of TEA was independent of the associated increase in agonist-induced contraction. These results demonstrate that: 1) volatile anesthetics induce relaxation of preconstricted isolated canine middle cerebral arteries and coronary arteries; 2) the middle cerebral arteries are more sensitive than coronary arteries to the dilating effects of volatile anesthetics; and 3) K$^+$ channel blockade potentiates the vasorelaxing effects of volatile anesthetics. (Key words: Anesthetics, volatile; enflurane, halothane, isoflurane. Artery: coronary, middle cerebral. Muscle, smooth: potassium channel. Pharmacology: tetraethylammonium.)

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VASODILATION ACCOMPANIES THE USE OF VOLATILE ANESTHETIC AGENTS THROUGH A DIRECT DEPRESSANT ACTION OF ANESTHETICS ON VASCULAR SMOOTH MUSCLE, or by an indirect attenuation of vasoconstrictor activity. The direct relaxant effect of inhalational agents may be more evident at the level of regional circulation. Volatile anesthetics alter cerebral blood flow in many animal species, as well as in humans. In the coronary circulation, the direct effects of volatile agents on arterial muscle are superimposed on their depressant effects on myocardium, decreasing cardiac work and oxygen demand. However, the volatile anesthetics also may be potent direct dilators of isolated coronary artery rings. In isolated, tetrodotoxin-arrested rat heart, halothane and isoflurane caused direct dilation of coronary resistance vessels without affecting oxygen consumption or extraction.

Because the functional state of vascular smooth muscle is dependent on the difference in electrical potential across the vascular smooth muscle cell membrane, alteration of transmembrane ionic channels offers a mechanism for the direct vasodilator effect of volatile anesthetics. The membrane potential of the vascular smooth muscle cell is mainly regulated by the flow of Ca$^{2+}$ and $K^+$ ions through specialized channels. Arterial smooth muscle tone is regulated by membrane potential primarily through the voltage-dependence of Ca$^{2+}$ influx. These channels play an important role in electromechanical coupling in cerebral and coronary arteries, because these vessels depend primarily on Ca$^{2+}$ influx to increase cytoplasmic Ca$^{2+}$ and activate contractile proteins during contraction. In isolated cerebral arteries, relaxation by halothane is associated with concentration-dependent membrane depolarization, suggesting electromechanical uncoupling. The mechanism by which halothane induces this apparent electromechanical uncoupling, i.e., causes membrane depolarization concurrent with vasodilation of vascular muscle cells, is not known. Because K$^+$ channel block would favor membrane depolarization, and Ca$^{2+}$ current suppression would induce vascular dilation, the uneven.

depression of both K⁺ and Ca²⁺ currents by halothane may help to explain the electromechanical uncoupling of vascular smooth muscle due to halothane. Activation of K⁺ channels will cause membrane hyperpolarization, reducing the influx of Ca²⁺ through voltage-dependent Ca²⁺ channels, and inducing vascular relaxation. K⁺ channels therefore act as an endogenous dilating mechanism to regulate vascular muscle tone. A decrease in the amplitude of both Ca²⁺ and K⁺ flux in the presence of volatile anesthetics has been demonstrated in the isolated coronary smooth muscle cell. However, the relative importance of these effects for the vasorelaxant effects of volatile anesthetics is unclear.

The purpose of this study was to determine the role of the tetroethylammonium-sensitive K⁺ channel in vascular relaxation induced by volatile anesthetics. Our hypothesis was that the blockade of K⁺ current would enhance vasoconstriction by agonists, but also may potentiate vasodilatory effect by anesthetics because of unopposed effect of anesthetics on Ca²⁺ current. Because anesthetic effects may vary in different vascular beds, both coronary and cerebral vessels were studied. In addition proximal and distal coronary arteries were studied to explore possible regional differences in response to volatile anesthetics. To eliminate potentially confounding indirect effects of anesthetics, an isolated vessel technique was used.

**Methods**

All experimental procedures strictly adhered to the standards of American Association for Accreditation of Laboratory Animal Care and all protocols were approved by the Animal Care Committee of the Medical College of Wisconsin.

Adult mongrel dogs of either sex were killed by exsanguination following anesthesia (sodium pentobarbital 30 mg/kg i.v.) and their brains and hearts were removed. The middle cerebral and coronary arteries were identified, carefully dissected and placed in physiological saline solution (PSS) of the following composition (in mM): NaCl 119, KCl 4.7; MgSO₄ 1.17; CaCl₂ 1.6; NaHCO₃ 27.8; NaH₂PO₄ 1.18; EDTA 0.026; glucose 5.5 and HEPES (4(2)-hydroxyethyl-1-piperazineethane sulfonic acid) 5. The vessels were cleaned of fat and connective tissue using a dissecting microscope and divided into rings of 2 mm in length. The vascular rings were mounted on tungsten triangles [the lower triangle being fixed and the other attached to a force transducer (Grass Model FT 10)] and suspended in jacketed, temperature controlled (37°C), tissue baths containing 15 ml of PSS aerated with a mixture of 93.5% O₂ and 6.5% CO₂. The pH, PCO₂ and PO₂ of the salt solution were monitored every 30 min and maintained constant at a pH of 7.38 to 7.42 and PCO₂ of 34 to 36 mmHg.

**Experimental Protocol**

The rings were progressively stretched to an optimal tension which was determined by preliminary length-tension studies using a standard concentration (40 mM) of KCl. The rings were then allowed to equilibrate for 90 min before conducting any experiments. Contractile responses were recorded continuously on a polygraph (Grass Model 7). The functional integrity of each ring was confirmed by the contractile response to 40 mM KCl in the bath medium. Following a further period of equilibration the rings were constricted to a stable plateau tension.

In the coronary arteries the relaxant responses to the anesthetics were measured in vessels previously exposed to the voltage-mediated constrictor KCl (40 mM). There is increasing evidence suggesting that coronary arteries respond differently depending on the size of the vessels. In order to study differential effects of volatile anesthetics, large (left anterior descending and left circumflex arteries) and small third degree branches were used. Beside KCl, the response of cerebral arteries to volatile anesthetics were studied after exposure of cerebral arteries to the receptor-mediated agonist prostaglandin F₂α (PGF₂α, 5 μM). In a random selection of vessels, the procedure was carried out in the presence of tetroethylammonium chloride (TEA, 20 mM). Because constrictor responses were enhanced in the presence of TEA, separate experiments were performed with vessels constricted with 15 μM PGF₂α in the absence of TEA. This constriction was comparable to that following 5 μM PGF₂α in the presence of TEA. This allowed exclusion of the possibility that augmented responses to anesthetics in the presence of TEA are caused by increased contraction.

**Anesthetic Delivery and Measurement**

Using separate vaporizers (Dragerwerk, Lubeck, Germany), each of three volatile anesthetic agents was bubbled in turn into the tissue baths. The order of administration of the anesthetics was randomized and the concentration of anesthetic vapor in the carrier gas was measured using a mass spectrometer (Marquette Electronics, Milwaukee, WI). Anesthetic concentrations in the tissue baths were determined by gas chromatography. Vessel rings were exposed to 0.45 ± 0.01, 0.40 ± 0.02 and 0.94 ± 0.02 mM isoflurane, halothane, and enflurane, respectively (mean ± SEM). These figures correspond to anesthetic concentrations of 2.1%, 1.35% and 3.25% and in the dog they represent minimum alveolar concentration values of 1.66 ± 0.04, 1.73 ± 0.09, and 1.67 ± 0.04 for isoflurane, halothane, and enflurane, respectively.

**Drugs**

Solutions of all drugs except volatile anesthetics were prepared daily. Prostaglandin F₂α and TEA were obtained
from the Sigma Chemical Company (St. Louis, MO). Isoflurane and enfurane were donated by Anaquest (Madison, WI), and halothane was obtained from Halocarbon Laboratories (North Augusta, SC).

**STATISTICAL ANALYSIS**

The responses of the middle cerebral and coronary arteries to inhalational anesthetics were expressed as a percentage relaxation (mean ± SEM) of the agonist-induced constriction to normalize the data. If more than one ring was used from the same animal with the same protocol, the mean response in these rings was used for statistical analysis. The responses of the vessels to each drug were compared by analysis of variance. When the F test showed significance, a t test was performed for comparison of means. A P value of < .05 was considered statistically significant.

**Results**

**CORONARY ARTERIES**

The vessel diameter of the small and large coronary arteries ranged from 350 to 550 μM and from 1,500 to 2,000 μM, respectively. The optimal resting tension was determined by initial response to 40 mM KCl at various levels of resting tension and ranged from 0.75 to 1.2 g and 1.8 to 3.0 g, for small and large arteries, respectively. Only vessels that had a prolonged, stable constriction in response to KCl were studied. Tetraethylammonium chloride (20 mM) had no effect on resting tension in either large or small coronary arteries. However, KCl (40 mM) produced greater constriction in TEA-pretreated than in non-TEA-pretreated small arteries (3.24 ± 0.33 vs. 3.99 ± 0.29 g) and large arteries (6.35 ± 0.27 vs. 7.46 ± 0.34 g) for non-TEA and TEA-pretreated arteries, respectively (P < .05). Figure 1 represents a typical tracing of a chart recording demonstrating the vasodilatory effect of volatile anesthetics on a single large coronary artery preconstricted with KCl in the presence (lower trace) and absence (upper trace) of 20 mM TEA. Summarized results for three anesthetics in small (fig. 2A) and large (fig. 2B) vessels also are shown. Enflurane produced significant (P < .05) relaxation of small and large coronary arteries preconstricted with KCl, while halothane produced significant relaxation only in large vessels. Isoflurane did not produce significant relaxation of coronary arteries preconstricted with KCl. The anesthetic-induced relaxation was enhanced when vessels were pretreated with TEA. Following treatment with TEA, all three anesthetics produced significant relaxation. There was no significant difference between small and large vessels in their response to anesthetics or in the effect of TEA on this response.

**MIDDLE CEREBRAL ARTERIES**

The vessel diameter of the middle cerebral arteries ranged from 500 to 800 μM. The optimal resting tension was 0.75–1.0 g. Only vessels that had a prolonged, stable constriction in response to KCl or PGF2α were studied. Tetraethylammonium chloride produced a transient increase in resting tension of variable amplitude, which returned to the pre-TEA level after 2–3 minutes. KCl (40 mM) produced greater constriction in vessels pretreated with TEA (2.28 ± 0.16 g vs. 3.17 ± 0.21 g, non-TEA pretreated vs. TEA pretreated, respectively; P < .05). Figure 3 represents a typical tracing of a chart recording demonstrating the vasodilatory effect of enflurane on the middle cerebral arteries preconstricted with KCl, with and without exposure to 20 mM TEA. Results for all three anesthetics are summarized in figure 4A. Volatile anesthetics produced significant relaxation of the canine middle cerebral arteries. In the isolated arteries preconstricted with KCl, enflurane produced significantly greater relaxation than either halothane or isoflurane. There was no difference in response between isoflurane and halothane. In vessels preconstricted with PGF2α and non-TEA-pre- treated, there was no difference among anesthetics. In
the case of each anesthetic, the relaxation was significantly greater in vessels pretreated with TEA. Also, in both KCl- and PGF$_{2\alpha}$-preconstricted arteries, halothane and enflurane produced significantly greater relaxation than isoflurane after pretreating arteries with TEA. Prostaglandin F$_{2\alpha}$ (5 μM) also produced greater constriction in vessels pretreated with TEA (1.4 ± 0.18 g vs. 2.13 ± 0.32 g, non-TEA-pretreated vs. TEA-pretreated, respectively, $P < .05$). Middle cerebral artery rings preconstricted with PGF$_{2\alpha}$ responded to anesthetics with greater relaxation than the vessels preconstricted with KCl (fig. 4B). This difference was statistically significant ($P < .05$) for isoflurane and halothane but not for enflurane. Cerebral arteries preconstricted with PGF$_{2\alpha}$ responded to anesthetics with significantly greater relaxation following pretreatment with TEA, with no difference in response between vessels preconstricted with KCl and PGF$_{2\alpha}$. Overall, the rings from middle cerebral arteries responded to anesthetics with greater relaxation than the rings from coronary arteries with and without the presence of TEA.

To exclude the possibility that the augmented response to anesthetics in the presence of TEA is caused by increased contraction, a group of vessels were preconstricted with 15 μM PGF$_{2\alpha}$, in the absence of TEA. This induced contractions were comparable to those induced by 5 μM PGF$_{2\alpha}$ in the presence of TEA (1.46 ± 0.2 g vs. 1.99 ± 0.26 g and 5 μM PGF$_{2\alpha}$ vs. 15 μM PGF$_{2\alpha}$, respectively). Enflurane-induced relaxations following 15 μM PGF$_{2\alpha}$ (18.2 ± 2.6%) were comparable to relaxations in the same vessels following PGF$_{2\alpha}$ 5 μM (17.4 ± 1.8%).

In some middle cerebral arteries, exposure to KCl (19 out of 48) and PGF$_{2\alpha}$ in the presence of TEA induced oscillations in tension of large amplitude at frequency of up to 5 cycles/min. With increased concentration of KCl and PGF$_{2\alpha}$, the amplitude of these oscillations decreased while their frequency increased. An example of this phenomenon is shown in figure 5. The results for all 19 vessels
volatile anesthetics also produce attenuation of arterial baroreflexes, and direct depression of sympathetic nerve activity, which contribute to the vasodilatory action of anesthetics. It is not entirely clear how alterations in cerebral or myocardial metabolism, endothelium-derived factors and vasoactive neurotransmitters and hormones interact with the direct actions of volatile anesthetics on the vascular muscle membrane to cause dilation. In the coronary circulation, the direct effects of halothane on coronary arterial muscle are superimposed on its effects in decreasing cardiac work and oxygen demand. Effects of volatile anesthetics on both cerebral and coronary flow depend in part on the preexisting cerebral or myocardial metabolic rate.

In vitro, halothane causes relaxation of isolated porcine coronary artery rings preconstricted with K⁺ or prostanoid to a greater degree than isoflurane. In studies on isolated coronary artery rings and isolated, tetrodotoxin-arrested rat heart, halothane caused direct dilation of coronary vessels without affecting oxygen consumption or extraction. Isoflurane has also been shown to be a potent dilator of isolated cerebral arteries. Compared with other vascular beds, large cerebral arteries contribute greatly to total cerebral vascular resistance. In mammals, it has been shown that under normal conditions, large arteries that supply the cerebral may contribute up to 60% of total resistance. Therefore a direct alteration of the diameter of these vessels by anesthetics may exert profound effect on cerebral vascular resistance and blood flow.

The exact cellular mechanism by which volatile anesthetics dilate vasculature is still unknown, although several studies have suggested either indirectly or directly that volatile anesthetics might act at the vascular muscle membrane, altering ionic fluxes. In cerebral arterial muscle, halothane caused membrane depolarization despite simultaneous vascular relaxation, suggesting uncoupling between membrane potential and vascular contraction. It also has been shown that halothane and isoflurane reduce the amplitude of K⁺ and Ca²⁺ currents in the vascular muscle membrane. However, the Ca²⁺ current was considerably more sensitive to blockade by volatile anesthetics. In general, contractile mechanisms in cerebral and coronary blood vessels appear to be more dependent on extracellular Ca²⁺ influx than on intracellular stores of Ca²⁺ and therefore, modulation of Ca²⁺ influx by volatile anesthetics represents a potential dilator mechanism. There is some evidence that extracellular Ca²⁺ may affect contraction of cerebral vessels more than extracerebral arteries. For example, the Ca²⁺ entry blocker nimodipine is a potent dilator of cerebral vessels but has a smaller effect on peripheral vessels.

Tetraethylammonium chloride inhibits current flow through the Ca²⁺-activated K⁺ channels. A TEA-sen-
sitive K⁺ current has been described in a variety of tissues including coronary and cerebral vascular smooth muscle. K⁺ channels play an important role in the regulation of arterial tone, likely acting as an endogenous dilating mechanism and by prevention of vasospasm. Activation of outward K⁺ current causes cell hyperpolarization, and inactivation of voltage-dependent Ca²⁺ channels leads to arterial relaxation.

The results of the present study demonstrate greater relaxation of KCl-preconstricted vessels by volatile anesthetics in the cerebral than in the coronary circulation. Middle cerebral artery rings preconstricted with PGF₂α demonstrated greater response to volatile anesthetics than the rings preconstricted with KCl. The responses to isoflurane, halothane, and enflurane were not equal, despite the use of comparable minimum alveolar concentration values. The relative potency in both vascular beds were as follows: enflurane > halothane > isoflurane. Tetrathyammonium chloride pretreatment was followed by an enhanced contractile response to both KCl and PGF₂α. The dilatation of both cerebral and coronary vessels to volatile anesthetics also was augmented by TEA and this effect was independent of the mechanism of vessel activation.

Tetrathyammonium chloride did not significantly alter tension in either coronary or cerebral vessels prestretched to the optimum tension, although in cerebral arteries, there was a transient increase in tension. However, the presence of a TEA-enhanced increase in tension developed on exposure of the pretreated vessels to increased K⁺ concentration or to PGF₂α. This suggests that the TEA-sensitive K⁺ current does not play an important role in regulating resting tension or that its role is minor. Another possible explanation is that a very small number of open K⁺ channels is required for maintenance of resting membrane potential, as suggested by Nelson et al. By contrast, when arteries are exposed either to KCl or to PGF₂α, the TEA-sensitive K⁺ channels are activated and may play a more important role, partially antagonizing the effect of the agonists by hyperpolarizing the cell.

Volatile anesthetics have been shown to depress both Ca²⁺ and K⁺ currents. The anesthetic-induced block of Ca²⁺ channel current would help to explain a direct dilatory effect on cerebral and coronary vasculature. On the other hand, the simultaneous block of K⁺ channel current by volatile anesthetics would favor membrane depolarization and therefore contraction due to opening
of voltage-dependent Ca\textsuperscript{2+} channels. However, the partial block of Ca\textsuperscript{2+} influx by volatile anesthetics would likely prevent the expected increase in cytoplasmic Ca\textsuperscript{2+} during this depolarization. Thus the contractile capacity of the cell may be impaired despite membrane depolarization. The enhancement of volatile anesthetic-induced relaxation in the presence of TEA suggests a complex interaction between anesthetic-induced Ca\textsuperscript{2+} and K\textsuperscript{+} channel blockade. The mechanism becomes further complicated because the depressant effect of volatile anesthetics on K\textsuperscript{+} current is likely to be potentiated by a decrease in Ca\textsuperscript{2+} current due to the known Ca\textsuperscript{2+} dependency of K\textsuperscript{+} current, which has been demonstrated in vascular smooth muscle.\textsuperscript{16,38}

In the majority of cerebral arteries, exposure to 20 nM TEA was accompanied by no change or a transient increase in tension. Transient increase in tension did not occur in coronary arteries. This could suggest a greater resting K\textsuperscript{+} current in cerebral than in coronary vasculature. Transient tetanic contraction of rabbit epicardial arteries by 10 nM TEA has been reported previously and explained as a result of summation of twitch responses due to spontaneous Ca\textsuperscript{2+} spike discharge.\textsuperscript{54} The same authors occasionally observed spontaneous periodic contractions during prolonged exposure to TEA. In the canine coronary arteries, we did not observe these oscillations in tension; however in the middle cerebral arteries, oscillations were present in a significant number of vessels following contraction of these vessels in the presence of TEA. Volatile anesthetics, particularly enflurane, were potent depressors of these oscillations. Presuming that the mechanism of these oscillations is the same as in rabbit coronary arteries, this would suggest that anesthetics are potent depressors of spontaneous Ca\textsuperscript{2+} spike discharge. Involvement of other mechanisms in vasorelaxation by anesthetics also has been suggested. These include alterations of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange in the cell membrane and changes in intracellular CGMP and/or cAMP.\textsuperscript{1,35} Alteration of contractile protein sensitivity to Ca\textsuperscript{2+} and effects on sarcoplasmic reticulum are also likely contributors to the relaxant effects of anesthetics.\textsuperscript{38}

In summary, the results of this study suggest that volatile anesthetics are more potent direct dilators of cerebral than coronary vascular smooth muscle. In addition, dramatic augmentation of dilator responses to volatile anesthetics followed a blockade of K\textsuperscript{+} current by TEA. Tetraethylammonium chloride also augmented contractile response to KCl and PGF\textsubscript{2\alpha}. Exposure of vessels not treated with TEA to an increased concentration of PGF\textsubscript{2\alpha} produced a contractile response comparable to that of lower concentration of PGF\textsubscript{2\alpha} in the presence of TEA. However, the relaxant response of these vessels to volatile anesthetics was comparable to responses in vessels not treated with TEA and contracted with a lower concentration of PGF\textsubscript{2\alpha}, suggesting that augmentation of response to volatile anesthetics is independent of increased constriction by agonists.

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References