Laboratory Investigations

Attenuation of contraction of isolated canine coronary arteries by enflurane and halothane

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Contraction of vascular smooth muscle such as that existing in coronary arteries is regulated in part by Ca^{++} entry into cells via Ca^{++} channels. Volatile anaesthetics are known to attenuate agonist-induced coronary artery constriction. The purpose of this experiment was to determine if 1.5 MAC concentrations of halothane or enflurane attenuated contractions evoked by activation of one type of Ca^{++} channel – the potential operator channel. In the current experiment, potential operator channels were activated by depolarizing isolated canine coronary artery rings with high concentration of K^+ , causing Ca^{++} entry and vessel contraction. Rings without endothelium were suspended for isometric force measurement in organ chambers containing aerated Krebs-Ringer solution. Maximum response to Ca^{++} in rings depolarized with K^+ was 120 ± 5 per cent in untreated versus 101 ± 3 per cent in rings treated with enflurane ($P < 0.01$). The maximum response was 123 ± 6 per cent in untreated versus 111 ± 5 per cent during halothane administration ($P < 0.05$). In contrast, nifedipine 10^{-9} M depressed maximum contractions from 114 ± 5 per cent to 37 ± 4 per cent ($P <$

0.01) and nifedipine 10^{-8} M depressed contractions to 30 ± 4 per cent ($P < 0.01$). In a further series of experiments, sustained contractions were depressed by continued administration of the anaesthetics, indicating no loss of anaesthetic effect with time. The results indicate that 1.5 MAC halothane and enflurane attenuate contractions of canine coronary arteries evoked by depolarization and Ca^{++} entry through potential operated channels. However, neither halothane nor enflurane exhibited the marked depressant effect exerted by nifedipine.

La réponse contractile des fibres musculaires lisses, comme celles qui existent au niveau des artères coronaires, est partiellement dépendante de l'entrée du calcium dans la cellule par des canaux calciques. Les anesthésiques volatils diminuent la contraction des fibres musculaires lisses vasculaires. Le but de cette étude était de vérifier si 1.5 MAC d'halothane ou d'enflurane atténue la réponse vasculaire suite à l'activation du canal calcique « potentiel dépendant ». Nous avons donc activé ce canal en dépolarisant des anneaux coronariens canins par une forte concentration de K^+ extracellulaire, il s'en est suivi une entrée de Ca^{++} dans la cellule et une contraction. Nous avons préparé des anneaux coronariens canins sans endothélium et avons étudié leur réponse dans des chambres tissulaires contenant une solution oxygénée de Krebs-Ringer à 37°C . La réponse maximale au Ca^{++} des anneaux dépolarisés par le K^+ fut 120 ± 5 pour cent pour les anneaux contrôles versus 101 ± 3 pour cent pour les anneaux traités à l'enflurane ($P < 0,001$). La réponse maximale fut de 123 ± 6 pour cent pour les anneaux contrôles versus 111 ± 5 pour cent après un traitement avec l'halothane ($P < 0,05$). Par contre, la nifédipine à la concentration de 10^{-9} M diminue la contraction de 114 ± 5 pour cent à 37 ± 4 pour cent ($P < 0,01$) et la nifédipine à la concentration de 10^{-8} M la réduit à 30 ± 4 pour cent ($P < 0,01$). Dans une autre série d'expériences, la tension maximale induite par la dépolarisation fut atténuée par l'administration prolongée des anesthésiques. Ces résultats montrent que l'enflurane et l'halothane à la concentration de 1.5 MAC diminue légèrement la contraction

Key words

ANAESTHETICS, VOLATILE: enflurane, halothane;
HEART: coronary artery;
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induite par la dépolarisation membranaire et l'activation du canal calcique « endothelium dépendant »; cet effet est cependant faible comparé à celui de la nifédipine, un antagoniste du calcium.

Episodic epicardial coronary artery constriction is an important cause of myocardial ischaemia in many patients with typical atherosclerotic coronary artery disease.^{1,2} Alleviation of constriction by vasodilator drugs with direct effects on coronary arteries such as Ca^{++} antagonists and nitrates can improve blood flow to the heart.²⁻⁴ Volatile anaesthetics are vasodilators and have direct effects upon epicardial coronary arteries. They have been shown to attenuate coronary artery constriction *in vivo* in pigs and contractions of isolated coronary arteries removed from dog, pig, and human hearts.⁵⁻⁷ The mechanism of this effect is not known and was the topic of this investigation. The study had two aims – to determine if 1.5 MAC halothane or 1.5 MAC enflurane attenuated contractions of canine coronary arteries evoked by depolarization and to determine if the relaxant effect equalled that exerted by nifedipine.

Contraction of vascular smooth muscle such as that existing in coronary arteries occurs when the concentration of Ca^{++} in the cytosol transiently increases.^{8,9} This increase originates from Ca^{++} release from intracellular stores and by entry of Ca^{++} through ion channels in the cell membrane.¹⁰⁻¹² One type of Ca^{++} channels are termed potential operated channels.¹⁰⁻¹² These channels open and allow influx of Ca^{++} in response to agonist-induced changes in cell membrane potential. (Agonists such as norepinephrine, serotonin, and endothelin also directly activate "receptor-operated" Ca^{++} channels and indirectly open "second messenger-operated channels": however, potential operated channels also appear to have an important role in constriction of human coronary arteries and are effectively blocked by drugs such as nifedipine, diltiazem, and verapamil.)¹³ In the current experiments, high concentrations of K^+ were used to depolarize isolated rings of coronary arteries and in this way activate potential operated channels while leaving receptor operated channels unstimulated.¹³ The question was to determine if the anaesthetics had an effect specific to potential operated channels.

The effects of halothane on depolarization-induced contractions are uncertain. Although halothane has been shown to attenuate contractions evoked by depolarization with K^+ in isolated pig coronary arteries,⁵ in contrast, in partially depolarized cerebral arteries of the cat, halothane elicited further contraction.¹⁴ Enflurane was investigated as little is known concerning the effects of this anaesthetic on coronary artery contractions. Isoflurane was not

investigated as it has previously been shown to lack effects on contractions induced by depolarization in isolated coronary arteries removed from both dogs¹⁵ and pigs.⁵

The effects of halothane and enflurane were also tested during prolonged contractions evoked by depolarization in order to determine if the anaesthetic effect was transient or sustained. In addition, the effects of nifedipine were examined in order to demonstrate the experimental preparations' responsiveness to a classical Ca^{++} antagonist drug. Nifedipine attenuates contractions evoked by depolarization in a wide range of vessels removed from many animal species including humans.¹³

Methods

Institutional Animal Care Committee approval was obtained and experiments were performed using twenty-two 18–28 kg dogs. They were anaesthetized with intravenous pentobarbital 35 mg·kg⁻¹ IV and their hearts excised. Left anterior descending and circumflex coronary arteries were dissected free, cut into rings 4–5 mm long, and cleaned of surrounding fat and connective tissue. Eight rings were removed from each heart. The endothelium was deliberately removed in all rings by gently rubbing the luminal surface with a wooden implement. (Volatile anaesthetics may have independent effects on endothelial function.^{15,16} In the current experiment, only vascular smooth muscle responses were studied; therefore, the endothelium was deliberately removed.) The rings were suspended in organ chambers filled with aerated (95 per cent O_2 – 5 per cent CO_2) modified Krebs-Ringer bicarbonate solution at 37° C at pH 7.40–7.49 of the following composition (mM): NaCl 119; KCl 4.9; CaCl_2 2.5; MgSO_4 , 1.2; KH_2PO_4 , 1.2; NaHCO_2 25; and glucose 4.1. The rings were attached to strain gauges (Harvard #52-9503) and isometric tension was recorded (Hewlett Packard 8802A amplifier, Gould 480 recorder).

Rings were placed at the optimal point of their length–tension relation by progressively stretching them until the contraction to KCl (20 mM), imposed at each level of distension, was maximal.¹⁷ Maximum force developed following a standard 40 mM KCl challenge was recorded and this response was used as a reference. Contractile and relaxation responses obtained during the experiment were compared with this reference contraction. Absence of endothelial function was tested by observing the lack of relaxation response of rings precontracted with 20 mM KCl to a single 10^{-6} M dose of acetylcholine.¹⁸ If the rings relaxed, they were replaced with rings removed from the same artery of the same heart.

The rings were allowed to equilibrate for 30 min. Paired

rings from the same artery from the same heart were studied in parallel. One ring served as control while the other was exposed to either halothane 1.3 per cent or enflurane 3.3 per cent (1.5 MAC in the dog¹⁹) added by vaporizer (Ohio Medical) to the gas mixture aerating the organ chambers. The concentrations of the anaesthetic in the aerating gas were adjusted to 1.5 MAC using a gas analyzer (Siemens Servo Gas Monitor 120). Anaesthetic concentrations in the perfusate were monitored by removing 400 μ l aliquots of Krebs solution, extracting the anaesthetic in hexane and measuring the concentrations using gas chromatography with electron capture (Hewlett Packard 1700C).²⁰

Isometric force developed by the rings was measured in the following experiments.

(a) The first experiment was designed to determine if halothane 1.5 MAC and enflurane 1.5 MAC each attenuated contractions evoked by Ca^{++} entry following depolarization induced with K^+ . Eleven dog hearts were studied. Two pairs of rings from each heart were used in the halothane experiments and two pairs of rings from each heart in the enflurane experiments. Sequentially increasing contractions of KCl were added to each organ chamber to achieve final K^+ concentrations ranging from 4.9 mM to 150 mM. Calcium concentration was maintained constant at 2.5 mM.

(b) The purpose of the second experiment was to determine if halothane 1.5 MAC and enflurane 1.5 MAC each attenuated contraction evoked by cumulative increasing concentrations of Ca^{++} in rings depolarized with K^+ . Six dog hearts were used. Two pairs of rings from each heart were used in the halothane experiments. Four of the hearts each provided two pairs of rings for use in the enflurane experiments. Rings were depolarized with 40 mM K^+ . Sequentially increasing concentrations of CaCl_2 were added by pipette to Ca^{++} free Krebs-Ringers solution in the chambers. The concentration of Ca^{++} was increased in steps from 0 to 5.20 mM.

(c) The third experiment was designed to determine the effects of halothane 1.5 MAC and enflurane 1.5 MAC on the time course of stable, sustained contractions induced by depolarization. Sustained stable contractions were induced for 60 min by adding K^+ 40 mM to the bath solution containing Ca^{++} 2.5 mM. Nine hearts each provided one pair of rings for use in the halothane experiments and eleven hearts each provided one pair of rings for use in the enflurane experiments. The anaesthetics were administered prior to and throughout the period of ring contraction.

(d) The purpose of the final experiment was to demonstrate the effects of nifedipine on contractions evoked by depolarization. Contraction was induced in rings from five hearts by adding KCl (10, 30, 70, and 150

FIGURE 1 Cumulative concentration response relationships of canine coronary artery rings exposed to increasing concentrations of K^+ in the presence of Ca^{++} 2.5 mM. Per cent contractions in the presence and absence of 1.5 MAC halothane and 1.5 MAC enflurane are shown. (Contractile responses are expressed as a percentage of the contractions obtained with 40 mM KCl.) Halothane and enflurane each depressed the contractions. Data are mean $\pm$ SEM (absence of SE bars indicates SE less than the dimensions of the data symbol).

mM) to the bath solution in the presence and absence of either 10^{-9} or 10^{-8} M nifedipine.

Drugs

Halothane was obtained from Ayerst and enflurane from Ohio Medical. Acetylcholine and nifedipine were obtained from the Sigma Chemical Company.

Statistics

The results are expressed as mean values $\pm$ SEM. In all experiments, n equalled the number of individual dog hearts used. Calcium chloride and potassium chloride concentrations are expressed as millimolar concentrations in the bath solution. Contractile responses are expressed as percentage of the contractions obtained to a standard 40 mM KCl challenge established at the beginning of the experiment. Relaxation responses are expressed at the beginning of the experiment. Relaxation responses are expressed as percentage decrease from the tension obtained following a standard 40 mM KCl challenge. Statistical evaluation was performed by paired Student's t testing and by comparing integrated areas under the dose-response curves using analysis of variance. Values were considered to be significant when P was less than 0.05.

Results

(a) The rings contracted in response to depolarization with increasing doses of KCl. Tension development was depressed both by halothane and by enflurane (Figure 1). In untreated rings, maximum tension was 123 ± 6 per cent of a reference contraction and in rings treated with halothane 1.5 MAC, maximum tension was 111 ± 5 per cent ($P < 0.05$). In untreated rings, maximum tension was 120 ± 5 per cent and in the presence of enflurane 1.5 MAC, it was 101 ± 3 per cent ($P < 0.01$).

FIGURE 2 Cumulative concentration response relationships of canine coronary artery rings exposed to increasing concentration of Ca^{++} in the presence of K^+ 40 mM. Per cent contractions in the presence and absence of 1.5 MAC halothane and 1.5 MAC enflurane are shown. (Contractile responses are expressed as a percentage of the contractions obtained with 40 mM KCl.) Enflurane but not halothane depressed the contractions. Data are mean $\pm$ SEM (absence of SE bars indicates SE less than the dimensions of the data symbol).

(b) Calcium chloride evoked concentration-dependent contractions of depolarized rings. Enflurane depressed tension development (Figure 2). In untreated rings, maximum tension was 144 ± 5 per cent and in the presence of enflurane tension was 107 ± 8 per cent ($P < 0.05$) of a control contraction. In these experiments, halothane had no effect on the contractile responses.

(c) Sustained ring contraction evoked by depolarization was depressed by both enflurane and halothane (Figure 3). In the halothane experiments, maximum tension generated by control rings was 115 ± 3 per cent and in rings treated with halothane the maximum tension was 97 ± 3 per cent ($P < 0.01$). In the enflurane experiments, maximum tension of untreated rings was 105 ± 4 per cent and in those receiving enflurane, it was 83 ± 4 per cent ($P < 0.01$).

(d) Nifedipine 10^{-9} M and 10^{-8} M depressed the contractile responses. The maximum tension generated by untreated rings was 114 ± 5 per cent. Nifedipine 10^{-9} M decreased the maximum tension to 37 ± 4 per cent ($P < 0.01$) and nifedipine 10^{-8} M decreased it to 30 ± 4 per cent ($P < 0.01$) (Figure 4).

Discussion

In the experiments reported here, both halothane 1.5 MAC and enflurane 1.5 MAC depressed contraction of canine coronary arteries evoked by Ca^{++} entry following depolarization with K^+ . The halothane effect was less marked, and was not observed in all the experiments. In contrast, nifedipine 10^{-9} M and 10^{-8} M exhibited profound attenuation of contractions, an effect previously established by other investigators.¹³

Depolarization of coronary artery rings with K^+ evokes contraction by permitting Ca^{++} to enter the cell via potential operated channels in the smooth muscle cell membranes.⁸⁻¹² (Depolarization may also enhance ex-

FIGURE 3 Tension-time curves of canine coronary rings precontracted by depolarization with 40 mM KCl in the presence of 2.5 mM CaCl_2 . Responses during control conditions and during administration of halothane 1.5 MAC (left) and enflurane 1.5 MAC (right) are shown. The anaesthetics were started at the plateau of the contraction and continued throughout the experiment. (Relaxation responses are expressed as percentage of contraction obtained following a standard 40 mM KCl challenge.) Both halothane and enflurane relaxed precontracted vessels. Data are mean $\pm$ SEM (absence of SE bars indicates SE less than the dimensions of the data symbol.)

change of Ca^{++} - Na^+ across the membrane, and Ca^{++} entering the cell following depolarization may also release Ca^{++} from intracellular stores.)⁸ Data from the current experiments indicate that Ca^{++} entry into the cell or the actions of Ca^{++} within the cell are modified by enflurane (1.5 MAC) and to a certain degree by halothane (1.5 MAC), and in this way contraction is inhibited.

The present data do not permit identification of enflurane's site of action, although inhibition of Ca^{++} entry into the cell via potential operated channels is one possible mechanism. Experimental models similar to that used here have been commonly used to identify compounds with Ca^{++} channel antagonist activity and in this model nifedipine, diltiazem, and verapamil have been shown consistently to attenuate contractions evoked by depolarization with K^+ .¹³ However, while these classical Ca^{++} antagonist drugs are known to lack significant effects within the cell, the same cannot be said for enflurane. The results of the current experiments imply that enflurane may, to a certain degree, inhibit Ca^{++} influx and/or may attenuate actions of Ca^{++} within the cell. It is likely that a number of sites of enflurane action exist. Multiple sites of action have been suggested to explain volatile anaesthetic effects in cardiac muscle.²¹

Halothane (1.5 MAC) attenuated contractions evoked by depolarization but the depressant effect was not great. However, it must be noted that although equi-MAC concentrations of the anaesthetics were used, the concentration of enflurane exceeded that of halothane.¹⁹ Halothane's relaxant effect persisted with time, sustained contractions being attenuated throughout the 60-min duration of the experiment.

Although halothane and enflurane are vasodilators and although halothane attenuates agonist-induced contrac-

FIGURE 4 Cumulative concentration response relationships of canine coronary artery rings exposed to increasing concentrations of K^+ in the presence of 2.5 mM Ca^{++} . Per cent contractions in the presence and absence of 10^{-9} M and 10^{-8} M nifedipine are shown. (Contractile responses are expressed as a percentage of contractions obtained following 40 mM KCl.) Nifedipine depressed the contractile responses. Data are mean $\pm$ SEM.

tions of coronary arteries, the results of the current study suggest antagonism of potential operated Ca^{++} channel function is not a major part of their mechanism of effect. These anaesthetics may act at other sites along the pathways that signal vascular smooth muscle contraction or upon the contractile process itself. Although both enflurane and halothane 1.5 MAC attenuate contractions of canine coronary arteries evoked by activation of potential operated Ca^{++} channels following depolarization with K^+ , neither anaesthetic exhibited the marked depressant effect on contractions exerted by nifedipine.

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