Laboratory Report

Volatile anaesthetics attenuate hypocapnia-induced constriction in isolated dog cerebral arteries

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Purpose: Hypocapnia causes cerebral arterial constriction, whereas volatile anaesthetics cause dilatation. The purpose of this study was to compare the direct effects of halothane, isoflurane and sevoflurane on hypocapnia-induced constriction of isolated cerebral arteries *in vitro*.

Methods: Basilar and middle cerebral arteries of mongrel dogs (n=11) were cut into rings and mounted for isometric tension recording in organ baths containing Krebs' bicarbonate solution, aerated with CO_2 5% and O_2 95% at 37°C. After constriction with 20 mM KCl, hypocapnia was induced by replacing the aerating gas with CO_2 2.5% and O_2 97.5% in the presence or absence of anaesthetics.

Results: Exposure of cerebroarterial rings to the hypocapnic gas produced sustained vasoconstriction (418 ± 19 mg), reaching a plateau within 10 to 15 min. Halothane (0.5, 1, 2 MAC) attenuated the hypocapnia-induced constriction ($P<0.05$). In contrast, isoflurane and sevoflurane attenuated this constriction only at 2 MAC ($P<0.05$). Attenuation by halothane was greater than that by isoflurane or sevoflurane at each concentration($P<0.05$). N^G-nitro-L-arginine (3×10^{-5} M) did not alter the contractile response to hypocapnia. When a similar degree of constriction was induced by addition of 10 mM KCl, halothane (1 and 2 MAC) preferentially attenuated the constriction induced by hypocapnia to a greater extent than that induced by 10 mM KCl ($P<0.01$)

Conclusion: Hypocapnia-induced vasoconstriction of isolated dog cerebral arteries precontracted with KCl is more susceptible to halothane than isoflurane or sevoflurane. This may account for the greater increase in cerebral blood flow during halothane than isoflurane or sevoflurane anaesthesia.

Objectif : L'hypocapnie provoque une vasoconstriction artérielle cérébrale alors que les anesthésiques volatils provoquent une vasodilatation. Cette étude visait à comparer les effets de l'halothane, de l'isoflurane et du sévoflurane sur la constriction induite *in vitro* des artères cérébrales par hypocapnie.

Méthodes : Des artères basilaires et cérébrales moyennes de chiens (n=11) ont été découpées en anneaux et montées dans des bains organiques contenant une solution de Krebs bicarbonatée, aérée avec du CO_2 à 5% et de l'O_2 à 95% à 37°C. Après constriction avec 20 mM de KCl, l'hypocapnie a été induite en remplaçant le gaz d'aération par du CO_2 à 2,5% et de l'O_2 à 97,5% avec ou sans anesthésique.

Résultats : L'exposition des anneaux d'artères cérébrales au mélange hypocapnique a produit une vasoconstriction soutenue (418 ± 19 mg) atteignant un plateau en 10 à 15 min. L'halothane (MAC 0,5 et 1,2%) a atténué la vasoconstriction induite par l'hypocapnie ($P<0,05$.) Par contre, en présence d'isoflurane et de sévoflurane, cette atténuation n'est survenue qu'à une concentration de 2% ($P<0,05$). Avec toutes les concentrations d'halothane, l'atténuation a été plus prononcée que celle produite par l'isoflurane et le sévoflurane ($P<0,05$). La Ng-nitro-L-arginine (3×10^{-5} M) n'a pas altéré l'effet contractile de l'hypocapnie. Avec un degré de constriction identique induit par l'addition de 10 mM de KCl, l'halothane (MAC 1 et 2) a atténué de façon préférentielle la constriction induite par l'hypocapnie à un degré plus important que la constriction induite par 10 mM de KCl ($P<0,01$).

Conclusions : La vasoconstriction induite par hypocapnie d'artères cérébrales canines isolées préalablement contractées avec du KCl est plus susceptible à l'halothane qu'à l'isoflurane et au sévoflurane . Ceci peut expliquer l'augmentation plus importante du débit cérébral pendant l'anesthésie à l'halothane que pendant l'anesthésie à l'isoflurane et au sévoflurane.

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IT is widely accepted that volatile anaesthetics possess the potential to increase cerebral blood flow (CBF) and intracranial pressure because of their cerebral vasodilating effects. However, the relative potencies of volatile anaesthetics to increase CBF vary. Most *in vivo* studies have demonstrated that the increase in CBF during isoflurane anaesthesia is less than that observed with halothane in humans[1,2] and experimental animals,[3–8] suggesting that halothane dilates cerebral vessels to a greater extent than does isoflurane.

The cerebrovascular dilatation caused by volatile anaesthetics may be affected by arterial carbon dioxide (CO_2) tension. Carbon dioxide is one of the most important physiological compounds involved in the regulation of CBF. Hypocapnia constricts the cerebral arteries and hence reduces CBF, which is thought to attenuate the vasodilatation caused by volatile anaesthetics. Although previous studies have demonstrated that halothane increases CBF to a greater extent than isoflurane during hypocapnia,[4,7] they do not necessarily indicate that halothane possess a stronger ability to dilate cerebral vessels during hypocapnia than isoflurane, since, in such *in vivo* experiments, the influence of the background anaesthesia and the inhibition of cerebral metabolism induced by the volatile anaesthetics tested could not be excluded. Furthermore, most studies of the effects of anaesthetics on cerebral circulation have been conducted in relation to their effects on the cerebral metabolic rate,[2–4,6,8] and they conclude that isoflurane does not increase CBF as much as halothane because of the marked reduction in cerebral metabolism caused by isoflurane. However, there is little information available about the direct effects of volatile anaesthetics on the cerebroarterial vasculature under hypocapnic conditions. Thus, it would be valuable to determine the direct effects of anaesthetics on the constriction of cerebral arteries mediated by a reduction in CO_2. To address this issue, the present *in vitro* study, devoid of the effects on cerebral metabolism and systemic haemodynamic changes caused by anaesthetics *per se*, was designed to compare the direct vasodilating effects of halothane, isoflurane and sevoflurane on hypocapnia-induced constriction of large dog cerebral arteries.

In addition, it is important to investigate the effects of nitric oxide (NO) on hypocapnia-induced constriction of cerebral arteries, since it has recently been demonstrated that blockade of NO synthase modulates the CBF response to a change in CO_2 tension *in vivo*.[9,10]

Materials and Methods

After receiving institutional approval, 11 adult mongrel dogs of both sexes, weighing 8–17 kg, were anaesthetized with 10 mg·kg^{-1} ketamine hydrochloride and 10 m·kg^{-1} pentobarbital sodium *iv*, and then killed by bleeding from the carotid arteries. The brain was removed rapidly, and the basilar and middle cerebral arteries (0.6–0.9 mm external diameter) were isolated, cleaned of surrounding tissues, and divided into 10–12 rings (3 mm long). Rings taken from the same dog were used for different experiments and as controls. Thus, the number of preparations indicates the number of rings obtained from the different dogs. Arterial rings with endothelium were suspended vertically between metal hooks in a 10-ml organ bath containing Krebs' bicarbonate solution maintained at 37.0 ± 0.3°C, and aerated with a mixture of CO_2 5% and O_2 95%. The upper end of one hook was connected to the lever of a force transducer (Nihon Kohden San-Ei Co, Tokyo, Japan) for recording isometric tension. The resting tension was adjusted to 2.0 g, which had been found to be optimal for inducing maximal constriction in a preliminary study. The constituents of the Krebs' bicarbonate solution were (in mM) NaCl, 118.2; KCl, 4.6; $NaHCO_3$, 24.8; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; dextrose, 10. Before the start of the experiment, the arterial rings were allowed to equilibrate for 60 to 90 min, during which time the bathing fluid was replaced every 10–15 min.

The contractile response to 30 mM KCl was evaluated first, and preparations were washed at least three times. To confirm endothelial integrity, endothelium-dependent relaxation was induced by substance P at a concentration of 10^{-8} M in rings which had previously been constricted with 3×10^{-6} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). After the response to substance P, 10^{-4} M papaverine was given to obtain maximal relaxation. Preparations in which substance P-induced relaxation was >60% of that induced by papaverine were considered to have an intact endothelium and were used for the study.[11] The arterial rings were then submaximally precontracted with 20 mM KCl and, after a plateau had been reached, hypocapnia was induced by replacing the aerating gas mixture with CO_2 2.5% and O_2 97.5%. After the contractile response of the arterial rings to hypocapnia had leveled off, the hypocapnic gas mixture was replaced with the normocapnic gas mixture. The tension that developed as a result of hypocapnia-induced constriction of the middle cerebral and basilar arteries was 384 ± 54 mg (n=11) and 416 ± 46 mg (n=11), respectively. There was no difference in the contractile response between the middle cerebral and basilar arteries. Therefore, either the middle cerebral or the basilar arteries was used in this experiment.

In the anaesthetic-treated groups, the bathing fluid was aerated continuously with a gas mixture containing halothane, isoflurane or sevoflurane 20 min before and during the hypocapnic period. The concentrations used were halothane 0.4%, 0.7% and 1.5%, isoflurane

0.6%, 1.2% and 2.4%, and sevoflurane 0.9%, 1.7% and 3.5%. These three concentrations were approximately equivalent to 0.5, 1 and 2 MAC in humans.[12-14] The contractile response to hypocapnia in the presence of the anaesthetics was compared with that in their absence (control). To determine whether endothelium-derived relaxing factor (EDRF)/NO modulated hypocapnia-induced constriction, some rings were treated with 3×10^{-5} M N^G-nitro-L-arginine (L-NA), a NO synthase inhibitor, for at least 20 min before the induction of hypocapnia.[15,16]

In the present study, the effects of anaesthetics on hypocapnia-induced constriction were examined in rings precontracted with KCl. To differentiate their effects on constriction induced by hypocapnia or by KCl, the inhibitory effects of halothane, isoflurane and sevoflurane (1 and 2 MAC) on the contractile response to hypocapnia and KCl were compared when the same degree of constriction as that induced by CO_2 2.5% was induced by further addition of 10 mM KCl.

The CO_2 tension, O_2 tension and pH of the bathing fluid were measured by a blood gas analyzer (Chiba Corning 286, Medfield, MA, USA). The volatile anaesthetics were delivered by a calibrated vaporizer (Fluotec 3, Isotec 3 and Sevotec 3, Ohmeda, England) and added to the aerating mixture. The concentration of the anaesthetic in the resulting gas mixture was monitored and adjusted using a calibrated anaesthetic gas monitor (Atom Co, Tokyo, Japan). In our previous study,[17,18] using the same method of application of volatile anaesthetics, the concentration of each anaesthetic in the bathing fluid, as measured by gas chromatography, reached equilibrium within five to seven minutes.

The drugs used were halothane (Hoechst Japan Limited, Osaka, Japan), isoflurane (Dainabott, Osaka, Japan), sevoflurane (Maruishi Pharmaceutical Co., Osaka, Japan), papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka, Japan) and N^G-nitro-L-arginine (Sigma Co., St. Louis, MO, USA). All results shown in this manuscript are expressed as the mean ± SEM. Statistical analysis was by Scheffe's F-test after analysis of variance, and Student's unpaired t-test, where appropriate. A P value of <0.05 was considered significant. In all experiments, n equals the number of dogs from which the rings were taken.

Results

Replacement of the normocapnic gas mixture with a hypocapnic mixture caused a gradual decrease in PCO_2 and an increase in pH of the bathing fluid, which stabilized within 10 min. The PO_2 was unchanged. The pH, PCO_2 and PO_2 of the bathing fluid during aeration with normocapnic and hypocapnic gas mixtures are summarized in Table I.

TABLE I The pH, PCO_2 and PO_2 of the bathing fluid aerated by normocapnic and hypocapnic gas mixtures

	pH	*PCO_2 (mmHg)*	*PO_2 (mmHg)*
Normocapnic gas mixture (CO_2 5%, O_2 95%, n=8)	7.42 ± 0.01	35.7 ± 0.6	630.6± 5.0
Hypocapnic gas mixture (CO_2 2.5%, O_2 97.5%, n=8)	7.62 ± 0.01*	21.4 ± 0.3*	641.7 ± 5.4

*Significant difference compared with the normocapnic gas mixture ($P<0.05$)

Constriction induced by 20 mM KCl averaged 1,153 ± 102 mg (n=11) in control rings, and 1,071 ± 136 mg (n=7), 1,029 ± 149 mg (n=7) and 937 ± 147 mg (n=7) in rings treated with halothane at 0.5, 1 and 2 MAC, respectively. Constriction induced by KCl averaged 1,249 ± 133 mg (n=7), 1,131 ± 126 mg (n=7) and 940 ± 203 mg (n=7) in rings treated with isoflurane at 0.5, 1 and 2 MAC, respectively, and 1,114 ± 98 mg (n=7), 1,149 ± 128 mg (n=7) and 963 ± 204 mg (n=7) in rings treated with sevoflurane at 0.5, 1 and 2 MAC, respectively. The volatile anaesthetics at concentrations up to 2 MAC did not affect vasoconstriction induced by 20 mM KCl.

The cerebral arterial rings gradually constricted in response to a decrease in the CO_2 concentration of the aerating gas mixture from 5% to 2.5%. The hypocapnia-induced constriction reached a plateau within 10 to 15 min (418 ± 19 mg, n=11). After stabilization, replacement of the hypocapnic gas mixture with a normocapnic mixture returned arterial tension to the control level (Figure 1, top). Treatment with L-NA did not alter the contractile response to hypocapnia; the mean values for constriction induced by hypocapnia in the presence and absence of 3×10^{-5} M L-NA were 385 ± 52 mg (n=8) and 409 ± 30 mg (n=8), respectively.

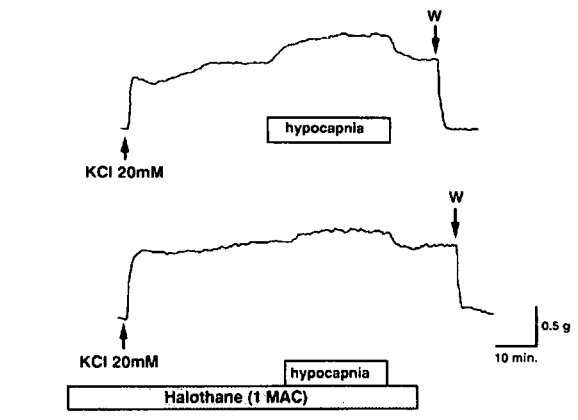

FIGURE 1 Representative recording of the change in tension induced by a hypocapnic gas mixture (CO_2 2.5%, O_2 97.5%) in the absence (top) and presence of halothane (1 MAC) (bottom).

Constriction induced by hypocapnia averaged 234 ± 14 mg, 214 ± 16 mg and 169 ± 13 mg in rings treated with halothane at 0.5, 1 and 2 MAC, respectively. Halothane attenuated the contractile response to hypocapnia at all concentrations tested (P <0.05, n=7) (Figure 1, bottom and Figure 2). In contrast, treatment with isoflurane or sevoflurane attenuated the response only at a concentration of 2 MAC (Figure 2). Constriction induced by hypocapnia averaged 334 ± 12 mg, 323 ± 12 mg and 277 ± 25 mg in rings treated with isoflurane at 0.5, 1 and 2 MAC, respectively (n=7), and 339 ± 13 mg, 331 ± 14 mg and 276 ± 15 mg in rings treated with sevoflurane at 0.5, 1 and 2 MAC, respectively (n=7). The inhibition of the contractile response to hypocapnia was greater in rings treated with halothane than in rings treated with isoflurane or sevoflurane at each concentration tested (P <0.05, Figure 2).

Addition of a further 10 mM KCl in arteries preconstricted with 20 mM KCl elicited a degree of cerebroarterial constriction similar to that induced by hypocapnia. The mean absolute values of constriction induced by the addition of 10 mM KCl and those induced by hypocapnia were 375 ± 66 mg (n=6) and 430 ± 75 mg (n=6), respectively. The constriction induced by hypocapnia or further addition of 10 mM KCl in the presence of anaesthetics is expressed as a percentage of the constriction in the absence of anesthetics (Table II). Halothane at concentrations of 1 and 2 MAC attenuated the constrictions induced by hypocapnia to a greater extent than constriction induced by 10 mM KCl (P <0.01, n=6). By contrast, there were no differences between the inhibition of the contractile responses to hypocapnia and to KCl in rings treated with isoflurane or sevoflurane at concentrations of 1 and 2 MAC (n=6).

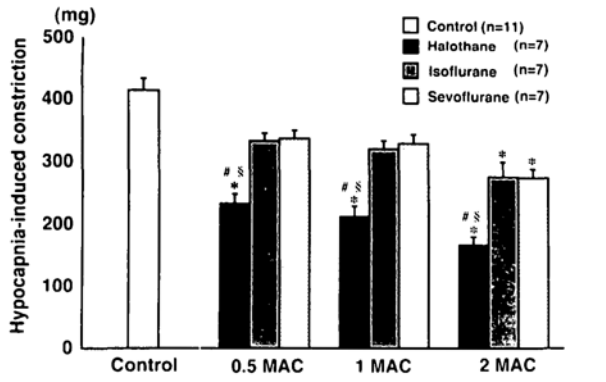

FIGURE 2 Effects of halothane, isoflurane and sevoflurane at concentrations of 0.5, 1 and 2 MAC on hypocapnia-induced constriction of dog cerebral arteries.

* P<0.05 *vs* control
\# P<0.05 *vs* isoflurane
§ P<0.05 *vs* sevoflurane at each concentration

Discussion

In the present study, we found that a reduction of CO_2 tension in the tissue bathing fluid caused sustained constriction of isolated dog cerebral arteries that were partially precontracted with 20 mM KCl. Halothane, even at low concentrations, strongly inhibited hypocapnia-induced vasoconstriction of cerebral arteries in a concentration-dependent fashion. By contrast, isoflurane and sevoflurane attenuated this constriction only at a high concentration. The attenuation caused by halothane was apparently greater than that caused by isoflurane or sevoflurane at all concentrations tested.

The present studies were designed to investigate the effects of volatile anaesthetics on hypocapnia-induced constriction of cerebral arteries precontracted with KCl. In a preliminary study, we found that hypocapnia-induced vasoconstriction in the resting state was small and unreproducible in some cerebral arterial strips, so the vasodilating effects of the anaesthetic agents could not be compared (data not shown). However, the tension induced by hypocapnia was greater and reproducible when the arterial rings were precontracted submaximally with KCl.

TABLE II Inhibitory effects of volatile anaesthetics on the contractile response to hypocapnia and further addition of 10 mM KCl in dog cerebral arteries preconstricted with 20 mM KCl

Anaesthetic	*Hypocapnia-induced constriction (%)*	*KCl (10 mM) -induced constriction (%)*	*P*
Halothane 1 MAC	56.8 ± 5.5	85.8 ± 4.8	0.003
Halothane 2 MAC	46.2 ± 7.0	78.9 ± 5.5	0.004
Isoflurane 1 MAC	88.9 ± 4.1	97.5 ± 3.8	0.152
Isoflurane 2 MAC	81.0 ± 2.5	86.6 ± 3.7	0.247
Sevoflurane 1 MAC	86.4 ± 7.1	88.1 ± 4.2	0.849
Sevoflurane 2 MAC	77.6 ± 5.8	85.1 ± 6.1	0.395

The constriction induced by hypocapnia or further addition of 10 mM KCl in the presence of anaesthetics is expressed as a percentage of the constriction in the absence of anaesthetics (n=6, each).

Therefore, we examined the influence of anaesthetics on hypocapnia-induced vasoconstriction in rings precontracted with 20 mM KCl. However, the inhibitory effects of the anaesthetics on hypocapnia-induced constriction that we observed might have been partly due to inhibitory actions on the effects of KCl. Therefore, we compared the inhibitory effects of these anaesthetics on the contractile response to hypocapnia and KCl when the same degree of constriction as that induced by CO_2 2.5% was induced by further addition of 10 mM KCl. Halothane, unlike isoflurane and sevoflurane, at concentrations of 1 and 2 MAC preferentially attenuated vasoconstriction induced by hypocapnia to a greater extent than that induced by KCl. The inhibitory effects of halothane on hypocapnia-induced constriction are thus unlikely to have resulted from attenuation of KCl-induced constriction. In support of our findings, it has been demonstrated that halothane (1.5%) relaxes isolated cat cerebral arteries in the resting state, but not when the arterial preparations are partially depolarized with high extracellular K^+.[19]

Recently, Reinstrup *et al.*[20] investigated the maximal contractile response to KCl and PGF_{2a} in ring segments of isolated human pial arteries in the presence of halothane or isoflurane during normocapnia and hypocapnia. They demonstrated that, during hypocapnia, halothane attenuated the contractile response to KCl and PGF_{2a} only at a concentration of 2 MAC, even though the inhibitory effects of halothane exceed those of isoflurane. These findings seem inconsistent with our results, in which halothane inhibited hypocapnia-induced contractions even at low concentrations (0.5 MAC). However, Reinstrup *et al.*[20] compared the inhibitory effects of anaesthetics on the maximal contractile response to KCl and PGF_{2a} during hypocapnia, whereas we compared the inhibitory effects of anaesthetics on hypocapnia-induced constriction in rings that were partially precontracted with KCl. These differences in experimental designs may be related to this discrepancy.

Sevoflurane is a relatively new volatile anaesthetic. Our present study reveals that sevoflurane affects hypocapnia-induced vasoconstriction only at a high concentration of 2 MAC, indicating that sevoflurane, like isoflurane, is a less potent inhibitor of hypocapnia-induced constriction of cerebral arteries than halothane. These findings are consistent with those reported in previous *in vivo* studies.[21,22] The effects of sevoflurane on CBF and cerebral metabolism are similar to those of isoflurane at equipotent concentrations in anaesthetized rabbits[21] and dogs.[22]

Hypocapnia-induced constriction of the cerebral arteries was not affected by the presence of L-NA, a NO synthase inhibitor, suggesting that the constriction is not derived from inhibition of NO. However, Iadecola[10] demonstrated in halothane-anaesthetized rats that application of L-NA inhibited the increase in CBF elicited by hypercapnia. It has also been reported that L-NA induces a dose-dependent decrease in normocapnic CBF and attenuates the hypercapnia-induced increase in CBF, but does not alter the hypocapnia-induced decrease in CBF in halothane-anaesthetized rats.[9] In contrast to observations *in vivo* experiments, hypercapnia-induced cerebroarterial relaxation was found to be independent of the presence of endothelium or L-NA in isolated dog[23] and rat[16] cerebral arteries *in vitro*. Whether or not NO participates in the regulation of CBF elicited by an alteration of CO_2 tension remains debatable but NO does not seem to be involved in cerebroarterial vasoconstriction induced by hypocapnia, when compared with hypercapnia and normocapnia. The inhibition by volatile anaesthetics of hypocapnia-induced constriction is unlikely to be related to the action of NO because neither removal of endothelium[24,25] nor the addition of L-NA[24] affected the vasodilatory effect of halothane and isoflurane on cerebral arteries. Further, volatile anaesthetics including halothane, isoflurane and sevoflurane have been widely demonstrated to inhibit the NO-cyclic guanosine 3,5-monophosphate (cGMP) pathway in isolated arterial rings.[18,26,27] The preferential inhibition by halothane of hypocapnia-induced constriction appears to be due not to a NO-cGMP pathway, but possibly to a direct action on vascular smooth muscle.[25]

Numerous *in vivo* studies have been performed to evaluate the influences of volatile anaesthetics on the cerebral circulation. It has been reported that CBF is increased by halothane (1 MAC) during hypocapnia, but not by equi-MAC isoflurane in rabbits.[4] In the cat, the CBF/$PaCO_2$ response curve was shifted to higher CBF values during halothane anaesthesia than with isoflurane.[7] These findings agree with those of our in vitro study. The alteration in CBF induced by an anaesthetic agent might be defined at least by two simultaneous effects[8,28]: 1. a direct vasodilatory effect on cerebral vascular smooth muscle, and 2, a secondary vasoconstriction as a result of cerebral metabolic depression caused by the anaesthetic. Thus, the net effect of an anaesthetic on CBF depends on the balance between its vasodilatory and cerebral metabolic depressant effects.[8,28] It has been suggested that the difference in effect on the cerebral circulation between halothane

and isoflurane is due to the greater cerebral metabolic depression caused by isoflurane than with halothane.[2,8,28] The present *in vitro* study clearly shows that the direct inhibitory effect of halothane on dog cerebral arteries exceeds those of isoflurane and sevoflurane, even though the metabolic effects of the anaesthetics may also influence the CBF.

In summary, we have demonstrated that hypocapnia-induced constriction of the cerebral arteries precontracted with KCl is more susceptible to halothane than to isoflurane or sevoflurane. Although these findings do not necessarily relate directly to the situation *in vivo*, this susceptibility may, in part, account for the greater increase in CBF during halothane anaesthesia than during isoflurane or sevoflurane anaesthesia.

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