Effects of sevoflurane on dopamine, glutamate and aspartate release in an in vitro model of cerebral ischaemia

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Release of excitatory amino acids and dopamine plays a central role in neuronal damage after cerebral ischaemia. In the present study, we used an in vitro model of ischaemia to investigate the effects of sevoflurane on dopamine, glutamate and aspartate efflux from rat corticostriatal slices. Slices were superfused with artificial cerebrospinal fluid at 34°C and episodes of 'ischaemia' were mimicked by removal of oxygen and reduction in glucose concentration from 4 to 2 mmol litre⁻¹ for <30 min. Dopamine efflux was monitored in situ by voltammetry while glutamate and aspartate concentrations in samples of the superfusate were measured by HPLC with fluorescence detection. Neurotransmitter outflow from slices was measured in the absence or presence of sevoflurane (4%). After induction of ischaemia in control slices, there was a mean (SEM) delay of 166 (7) s (n=5) before sudden efflux of dopamine which reached a maximum extracellular concentration of 77.0 (15.2) µmol litre⁻¹. Sevoflurane (4%) reduced the rate of dopamine efflux during ischaemia (6.90 (1.5) and 4.73 (1.76) µmol litre⁻¹ s⁻¹ in controls and sevoflurane-treated slices, respectively; P<0.05), without affecting its onset or magnitude. Excitatory amino acid efflux was much slower. Ischaemia-induced glutamate efflux had not reached maximum after 30 min of ischaemia. Basal (pre-ischaemic) glutamate and aspartate efflux per slice was 94.8 (24.8) and 69.3 (31.5) nmol litre⁻¹ superfusate (n=4) and was not significantly reduced by 4% sevoflurane. Ischaemia greatly increased glutamate and aspartate efflux (to a maximum of 919 (244)% and 974 (489)% of control, respectively). However, ischaemia-induced efflux of both glutamate and aspartate was significantly reduced by 4% sevoflurane (P<0.001 for glutamate, P<0.01 for aspartate). In summary, sevoflurane may owe part of its reported neuroprotective effect to a reduction of ischaemia-induced efflux of excitatory amino acids and, to a lesser extent, dopamine.

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Sevoflurane is a volatile anaesthetic suitable for both induction and maintenance of anaesthesia.¹ Clinically, it produces a pattern of rapid recovery with little excitation on emergence.² Its capacity to increase cerebral blood flow³ whilst preserving cerebral autoregulation⁴ makes it an attractive agent for the preservation of neuronal function.

There is some evidence from animal studies that sevoflurane is neuroprotective in models of cerebral ischaemia.⁵ Since global ischaemic insults, and even frank infarcts, can occur in a theatre setting, a neuroprotective anaesthetic is of considerable clinical interest.

During cerebral ischaemia, a cascade of events occur that result in neuronal death. One of the earliest of these is the release of neurotransmitters, in particular the excitatory amino acids.⁶ Reduction of transmitter release thus represents a possible mechanism by which drugs may exert a neuroprotective effect.

Previous work in this laboratory has examined transmitter efflux in an in vitro model of cerebral ischaemia based on brain slices.⁷ We have found that various recognized neuroprotective agents reduce neurotransmitter efflux. In particular, we recently found that halothane slowed
ischaemia-induced dopamine efflux. In the present study we examined the effects of sevoflurane on the efflux of dopamine, glutamate and aspartate.

Materials and methods

Preparation of brain slices

Male Wistar rats (150–300 g) were killed by cervical dislocation. No prior neuroprotective agents were administered. The cranial vault was opened and the brain excised while irrigated with ice-cold (−1 to +1°C) oxygenated artificial cerebrospinal fluid (aCSF) throughout. Coronal corticostriatal slices (350 μm thick: −0.7 to +1.3 mm vs bregma) were prepared using a Campden 752M vibratome and transferred to a ‘slice saver’ containing 500 ml continuously oxygenated (95% oxygen/5% carbon dioxide) aCSF maintained at room temperature (24°C) where they were incubated for 1–4 h to allow recovery from the trauma induced by preparation.

Standard aCSF (pH 7.4) was used, consisting of (mmol litre−1) NaCl (126.0), KCl (4.0), KH2PO4 (1.4), MgSO4 (1.3), CaCl2 (2.4), NaHCO3 (26.0), (+)-glucose (4.0) and ascorbic acid (0.4). The glucose concentration was reduced (approximately 1.7 MAC10) throughout the experiment (during both pre-incubation in maintenance aCSF and single slices were placed in the chamber and perfused with aCSF at 400 ml h−1. For excitatory amino acid experiments, superfusion with ‘ischaemic’ aCSF was initiated for 30 min. Sevoflurane was applied to the brain slices at 4% (approximately 1.7 MAC10) throughout the experiment (during both pre-incubation in maintenance aCSF and imposition of ischaemia) via a calibrated Blease Datum vaporizer.

Detection of dopamine efflux

Ischaemia-induced dopamine efflux was measured using fast cyclic voltammetry. Carbon fibre microelectrodes, 7 μm in diameter, were positioned in the dorsolateral zone of the striatum, under micromanipulator control. Auxiliary (stainless steel) and reference (Ag/AgCl) electrodes were located at convenient points in the slice chamber where they would not obstruct the voltammetric or d.c. potential electrodes. Voltammetric scans (−1.0 to +1.4 V compared with reference electrodes, 480 V s−1) were performed every 4 s with a Millar Voltammetric Analyser (PD Systems, West Molesey, UK). A ‘sample-and-hold’ record of current at the peak dopamine oxidation voltage (+0.6 V compared with reference electrodes) was displayed on a chart recorder while entire voltammetric scans were recorded on a digital storage oscilloscope (Nicolet 310 Series) and saved on to floppy disk. At the end of the experiment, the carbon fibre microelectrodes were calibrated by standard flow injection analysis (400 ml h−1) in solutions of dopamine (500 μl of 100 μmol litre−1).

Three dopamine efflux variables were measured: the time from initiation of ischaemia to the onset of dopamine efflux (ton, in s), the maximum extracellular dopamine concentration (DAmax, in μmol litre−1) and the mean rate of dopamine efflux from ton to DAmax (dDA/dt, in μmol litre−1 s−1).

Detection of excitatory amino acid efflux

At 5 min intervals throughout the experiment, a 1 min sample (5 ml) of slice superfusate was collected into glass vials and frozen until analysed for glutamate and aspartate content. Glutamate and aspartate were determined using a modification of the HPLC method of Smolders and colleagues.11 The method is based on the derivatization of amino acids using o-phthalaldehyde (OPA). A stock solution of 120 mg OPA in 6 ml methanol was prepared. Derivatizing reagent was prepared by mixing 0.6 ml OPA solution with 5.4 ml borate buffer (pH 9.0) and 60 μl 2-mercaptoethanol. Equal volumes (50 μl) of superfusates collected from slices and derivatizing reagent were mixed at room temperature (24–26°C) and, exactly 2 min later, 50 μl were injected into the chromatograph.

The OPA derivatives were separated on a 100×3.2 mm i.d. HIRPB column (HiChrom, Reading, UK) eluted with 10% HPLC-grade acetonitrile in 0.1 M sodium acetate buffer (pH 6.0) at 1 ml min−1 with an Altex 110 pump (Beckman, High Wycombe, UK). The compounds were detected on a Perkin–Elmer LS-4 fluorimeter (Beaconsfield, UK) with the excitation monochromator set at 350 nm and the emission set at 450 nm. Peaks were recorded on an analogue recorder or digitally using a 16 bit A/D converter (Jones Chromatography, Hengoed, UK) in an ICL M55 computer. Four minutes after the injection, the column was washed with 90% acetonitrile in water for 1 min, using a second Altex pump and an electronic valve actuator (Jones Chromatography) controlled from the computer. The column was equilibrated with eluent for 5 min before the next injection. Calibration curves were generated using standard solutions in water containing 125–1000 nmol litre−1.
glutamate and aspartate and data were expressed as nmol of amino acid litre⁻¹ superfusate per slice.

For estimation of variability, calibration solutions were prepared in the range 1±0.031 μmol litre⁻¹, plus blank. Estimates of the intra- and inter-assay precision and accuracy values were obtained by quintuplicate analyses of solutions containing 1, 0.5, 0.2, 0.1, 0.05 and 0.02 μmol litre⁻¹ of aspartate and glutamate. Intra-assay coefficients of variation of replicate analyses of glutamate and aspartate concentrations were between 2 and 6%. The lower limit of quantification was taken as 0.05 μmol litre⁻¹ as coefficients of variation for samples at 0.02 μmol litre⁻¹ were >20%.

Drugs and chemicals
All chemicals used to make the aCSF were of standard AnalaR grade from BDH Lab supplies (Poole, UK). Dopamine was obtained from Sigma (Poole, UK). Sevoflurane was a gift from Abbott Laboratories Ltd.

Data analysis
The effects of sevoflurane on ischaemia-induced excitatory amino acid efflux were calculated as percentages of presischaemia values and were compared with control by two-way analysis of variance (ANOVA). The effects of sevoflurane on basal (pre-ischaemic) glutamate and aspartate efflux were analysed by unpaired t-tests. The effects of sevoflurane on individual measures of dopamine efflux were tested using paired t-tests.

Results
Induction of a period of ischaemia (hypoxia/hypoglycaemia) in slices at 34°C resulted in a consistent pattern of striatal dopamine efflux in untreated control tissue. After an initial period of quiescence lasting 2–3 min, there was a sudden efflux of dopamine, typically reaching a peak extracellular concentration of 50–100 μmol litre⁻¹ within 20 s before falling slowly back towards baseline over a period of several minutes. Figure 1 shows a representative profile of dopamine efflux in control and sevoflurane-treated striatal slices.

Although the onset of dopamine efflux appeared longer in 4% sevoflurane (mean (SEM) t₀ 166 (7) s in controls, 211 (64) s in sevoflurane-treated slices; n=5), this was not significant (note the truncated x-axis in Figure 1). Sevoflurane did not significantly reduce the maximum level of dopamine efflux (DAmax 77.0 (15.2) and 67.8 (12.4) μmol litre⁻¹ in controls and sevoflurane-treated slices, respectively). However, the rate of dopamine efflux was significantly lower in slices exposed to sevoflurane (dDA/dt 6.90 (1.5) and 4.73 (1.76) μmol litre⁻¹ s⁻¹ in controls and sevoflurane-treated slices, respectively; P<0.01).

Basal excitatory amino acid efflux in the superfusate was not significantly different in control slices and those treated with 4% sevoflurane (Table 1). The effects of ischaemia on excitatory amino acid efflux were very different from those on dopamine efflux. Figure 2 shows the effect of sevoflurane on ischaemia-induced glutamate efflux. After imposition of ischaemia, there was a significant (P<0.001) time-dependent increase in glutamate efflux over the 30 min course of the ischaemic episode. This was significantly reduced in the presence of 4% sevoflurane (P<0.001; two-way ANOVA).

A similar profile was observed with aspartate. The effect of sevoflurane on ischaemia-induced aspartate efflux is shown in Figure 3. As with glutamate, there was a substantial increase in aspartate efflux over the duration of the ischaemic episode. This was essentially abolished in the presence of 4% sevoflurane (P<0.01; two-way ANOVA).

Discussion
The role of excitatory amino acids in ischaemic damage is well established. Efflux of glutamate and/or aspartate is an early event in a cascade that leads to neuronal death and drugs that block the postsynaptic actions of excitatory amino acids at N-methyl-D-aspartate (NMDA) or alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors have been shown to be neuroprotective in
ischaemia. However, glutamate is only one of many transmitters released in ischaemia. Recent reports have suggested that dopamine released in ischaemia may also mediate neuronal damage, at least in the striatum and at least partly through an interaction with D2 receptors.

An alternative approach to neuroprotection, rather than blocking postsynaptic actions, is to prevent the efflux of neurotransmitter. Studies by our group have shown that ischaemia-induced dopamine efflux may be attenuated or delayed by several agents or strategies with neuroprotective potential. These include blockers of calcium and sodium channels, NMDA antagonists and hypothermia.

Efflux of glutamate and aspartate in ischaemia is sometimes held to be mediated by reversal of the glutamate carrier. The mechanism of dopamine efflux is less clear. Whilst at least one group has suggested that it too occurs through reversal of the transporter, we found no effect of dopamine uptake inhibitors on ischaemia-induced dopamine efflux and thus suggested that its primary mode of release was exocytotic.

The possibility that general anaesthetics are neuroprotective is widely promoted. Although ketamine is known to be neuroprotective, presumably because of its capacity to block NMDA receptors, it is possible that others with less overt actions may also be protective. We have recently shown that halothane is able to slow dopamine efflux in this model.

In the present study, we found that sevoflurane not only slowed ischaemia-induced dopamine efflux in a manner similar to halothane but also reduced release of glutamate and aspartate. The effect on aspartate was particularly striking. However, since aspartate binds to NMDA receptors with approximately a tenth of the affinity of glutamate, such a large reduction in its release is likely to have less neuroprotective consequences than the diminution of glutamate release.

It should be said that in measuring dopamine efflux by voltammetry, we have examined the effect of sevoflurane on ischaemia-induced transmitter release from striatal terminals, whereas with the method for investigating excitatory amino acid efflux, we have examined transmitter release from cells and synaptic terminals in both the cortex and striatum. It would be interesting to discover if the differential effect of sevoflurane on maximal ischaemia-induced transmitter release lies in this different neuroanatomical source of transmitter or whether it reflects true differences in the mode of action of the anaesthetic on the different transmitter systems per se. Such questions might be resolved by electrode-based techniques, but these have not yet reached the technological level required for such studies.

Sevoflurane has been reported to be an effective neuroprotective agent in cerebral ischaemia. For instance, Werner and colleagues found that sevoflurane improved outcome after a focal ischaemic insult in rats. Sevoflurane also expedites the recovery of brain energy metabolism, relative to halothane, in global ischaemia.

In common with other volatile anaesthetics, sevoflurane has many biochemical actions. It blocks nicotinic receptors whilst enhancing the effects of agonist stimulation at \( \gamma \)-amino-butyrate (GABA)\(_A\) and GABA\(_B\) receptors. Recently, Li and Pearce showed that halothane enhanced the effects of GABA at GABA\(_A\) receptors by slowing the dissociation of the agonist; they suggested that this may be a major mode of action of other volatile anaesthetic agents. Also of interest in the context of the present report, a recent study found that sevoflurane enhanced glutamate uptake by astrocytes. Such an effect could contribute to the decreased efflux not only of glutamate observed here but also of aspartate, which is also a substrate for the transporter.

The volatile anaesthetics have also been shown to activate potassium channels whilst blocking sodium channels.
channels in rat brain slice preparations. We have previously shown that sodium channel blockade slows ischaemia-induced dopamine efflux in striatal slices, so it is possible that such a mechanism underlies some of the effects of sevoflurane observed here.

Throughout this study, sevoflurane administration was initiated before the ischaemic insult. Although this has little bearing on the treatment of stroke, where neuroprotection can only be administered in a post-ictal manner, such a circumstance would be analogous to that found in the operating theatre, where an ischaemic episode might occur under anaesthesia. Surgery for carotid endarterectomy or coronary artery bypass grafting carries a significant risk of cerebral ischaemia, so there is a role for prior administration of appropriate neuroprotectants. The results of the present study show that sevoflurane, at a clinically relevant concentration (approximately 1.7 MAC), can reduce the efflux of neurotoxic transmitters under such conditions and thus may be neuroprotective.

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