Synergistic antinoceptive interaction between sevoflurane and intrathecal fentanyl in dogs

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Summary
This study determined the nature of the antinoceptive interaction between sevoflurane and intrathecal fentanyl on somatosympathetic reflexes in anaesthetized dogs. Afferent Aδ- and C-fibre-mediated somatosympathetic reflexes, evoked by supramaximal electrical stimulation of tibial nerves, were recorded from renal sympatoceptive nerves. The effect of fentanyl alone, administered intrathecally (i.t.) in incremental doses from 2 to 64 μg, was compared with the effect of the same doses during the administration of 1.5% sevoflurane. The mean ED50s for the depressant effect of fentanyl (i.t.) on Aδ and C reflexes were 35.6 μg and 14.2 μg while 1.5% sevoflurane, when administered alone, depressed them by 15.5% (P < 0.05) and 27.5% (P < 0.01) respectively. During the administration of 1.5% sevoflurane, the mean ED50 of fentanyl (i.t.) for the depression of Aδ and C reflexes were reduced by 76% and 75%, to 8.5 μg and 3.5 μg respectively. The combined antinoceptive effects of sevoflurane and intrathecal fentanyl were not additive but exhibited a high degree of synergistic interaction. (Br. J. Anaesth. 1998; 80: 800–806)

Keywords: anaesthetics volatile sevoflurane; analgesics opioid fentanyl; reflexes somatosympathetic; interactions (drug) synergy; dog

In the 1970s it was shown that the intrathecal (i.t.) administration of opioids could provide pain relief.12 These studies contributed to our understanding of the regional role of opioid receptors, which are not only in supraspinal centres but also, for example, in the dorsal horn of the spinal cord on the terminals of small-diameter primary afferent fibres.3 4 Recent work has suggested that the spinal cord is one of the principal sites of action of inhalation anaesthetic agents,6 9 and this has been summarized by Eger and associates.10 Although previous studies have shown that opioids administered spinally can reduce the minimum alveolar concentration (MAC) of inhalation agents in humans and animals11–14 the nature of their combined effect, that is whether additive or interactive, has not been investigated. We have tested the hypothesis that an inhalation anaesthetic and an opioid administered intrathecally act synergistically to depress nociceptive responses.

Single supramaximal electrical stimuli applied to somatic nerves evoke two bursts of activity in sympatoceptive nerves because of the activation of both afferent myelinated (group III or Aδ) and unmyelinated (group IV or C) fibres.15 These reflexes are called “somatosympathetic reflexes”. They can be used to evaluate the antinoceptive effects of drugs16 and are important in the management of anaesthetized, paralysed and ventilated patients. Unsynchronized stimulation of Aδ and C fibres provides the variable nociceptive responses to surgical trauma. A controlled input by electrical stimulation of a specific nerve allows the relative quantitative effects of drugs on the Aδ and C components of nociception to be compared. In the present study, Aδ- and C-fibre mediated somatosympathetic reflexes were used as nociceptive indices to examine the type of interaction between sevoflurane and fentanyl administered intrathecally in anaesthetized dogs.

Materials and methods

GENERAL PROCEDURE
The study was approved by the UK Home Office (Licence PPL 90/00852). We studied 16 beagles weighing 13.5–17 kg. Background anaesthesia was induced with methohexitone (Eli Lilly, UK) 10–15 mg kg⁻¹ i.v. and maintained with 1% α-chloralose (Sigma, Poole, Dorset, UK) in an initial bolus dose of 30 mg kg⁻¹ i.v., followed by a continuous infusion of 15–20 mg kg⁻¹ h⁻¹ i.v. The trachea was intubated and the lungs ventilated with oxygen-enriched air (HV 2000, SLE Ltd, Croydon, UK). Muscle paralysis was maintained using suxamethonium (Wellcome, Crewe, Cheshire, UK) 10 mg i.v. every 20–30 min. The cephalic vein in the right foreleg was cannulated for recording arterial pressure and sampling blood. Oesophageal temperature was measured with a thermistor (Yellow Springs Instruments, OH, USA) and maintained at 37–39°C by a heater in the operating table. Arterial blood gas tensions and pH were determined regularly using a blood gas analyser (Radiometer ABL 3, Copenhagen, Denmark). pH and PaCO2 were kept within normal limits, that is, 7.30–7.40 and 4.8–5.6 kPa respectively, and the PaO2 at 22.7–26.7 kPa; all three were kept close

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Sevoflurane, fentanyl and somatosympathetic reflexes to the control values in each preparation by small changes in the $F_{10}$ and ventilation, and occasional administration of small amounts of sodium bicarbonate. Heart rate and mean arterial blood pressure (MAP) were recorded continuously (Maelab ADInstruments, ADInstruments Pty Ltd, Australia).

Each preparation was maintained in a left lateral position. The tibial nerve in the right hindleg was exposed in the upper to middle part of the thigh. A portion was dissected free of the surrounding tissues and a short section ($\approx 2$ cm) was desheathed, cut distally and mounted on silver–silver chloride electrodes for stimulation. The renal sympathetic nerves were exposed retroperitoneally alongside the renal artery; a single fascicle was desheathed and cut distally, near the kidney, and mounted on silver–silver chloride electrodes for recording sympathetic activity. Prepared nerves were immersed in mineral oil pools. For intrathecal injection of drugs a 22 gauge Y-can cannula (Wallace, Essex, UK) was inserted into the subarachnoid space through the dura exposed by laminectomy at L2–L3. Sevoflurane (Abbott Laboratories Ltd, Queensborough, UK) was administered with a Blease vaporizer (Chesham, Bucks, UK). The preparation was allowed to stabilize for at least 30 min later to ensure that the preparation was stable.

Three groups of data were obtained for spontaneous sympathetic activity, and $A$ and $C$ somatosympathetic reflexes evoked by tibial nerve stimulation: (1) the effect of intrathecal (i.t.) fentanyl alone; (2) the effect of sevoflurane alone, principally to determine accurately the ED$_{50}$ concentrations for both $A$ and $C$ reflexes and also the effect of a concentration of 1.5%; (3) the effect of fentanyl (i.t.) during administration of 1.5% sevoflurane.

MEASUREMENTS OF EVOKED AND SPONTANEOUS SYMPATHETIC ACTIVITY

Single supramaximal electrical stimuli (frequency 0.33 Hz, intensity 30 V, duration 0.5 ms) were applied to the tibial nerves by a stimulator (Grass S88, Quincy, MA, USA) with a matching isolation unit (Grass 478A). Efferent activity in renal sympathetic nerves was processed through a preamplifier (Tektronix type 122) and displayed on a dual-beam oscilloscope (Tektronix type 565). Spontaneous sympathetic activity was subjected to full-wave rectification and then integrated with a time constant of 100 ms (Neurolog NL 90). The evoked somatosympathetic responses in renal sympathetic nerves were averaged ($\times 16$), rectified, and integrated using a neurolog system (NL 90, Digitimer, Welwyn Garden City, UK). Directly recorded and processed signals were displayed on an oscilloscope and also, when required, as “hard” copy on a thermal recorder (Gould 1602). The total electrical activity in the integrated signals of the rectified and averaged evoked sympathetic responses, and also the spontaneous sympathetic outflow during 20-s periods, was measured in arbitrary units. Data were based on the average of three sets of measurements at each point in each preparation and were expressed as a percentage of control values.

EXPERIMENTAL DESIGN

The preparation was allowed to stabilize for at least 30 min after completion of surgery, before control measurements were made; these were repeated 30 min later to ensure that the preparation was stable. In another five preparations, the ED$_{50}$s for the concentrations of sevoflurane that reduced the $A$ and $C$ reflex response to radial nerve stimulation by 50% were determined. This was done by first obtaining control values for these reflexes in the absence of sevoflurane, which was then administered to an equilibrated end tidal concentration of 2.6%. (This had been shown in a previous study to depress the mean C response to radial nerve stimulation by 50%.) Then, in the present study the “up-and-down” method with step changes of 0.2% was used to titrate accurately the ED$_{50}$ concentration for depression of the $C$ response to radial nerve stimulation. The end tidal concentration of sevoflurane was then increased to 3.7% (which had been shown previously to be the ED$_{50}$ for the $A$ response to radial nerve stimulation), and the accurate ED$_{50}$ for depression of the $A$ response to tibial nerve stimulation was determined using similar methods. These values, together with the ED$_{50}$ of intrathecal fentanyl alone, were used to construct isobolograms for the combined effects of the two drugs (fig. 5).
In another six preparations, control values were first obtained for spontaneous sympathetic activity and Aδ and C somatosympathetic reflexes evoked by tibial nerve stimulation, in the absence of both the inhalation agent and intrathecal fentanyl. Sevoflurane was then administered in a concentration of 1.5%. (A previous study had shown that sevoflurane at this concentration reduced C reflexes in response to radial nerve stimulation by around 30%, and also that its effects on somatosympathetic reflexes remained unchanged during observations lasting more than 3 h.) The effect of an equilibrated concentration of 1.5% sevoflurane on Aδ and C sympathetic reflexes evoked by stimulation of the tibial nerve was observed, stability of the preparation confirmed for 20 min, and new “control” data obtained showing the depressant effect of 1.5% sevoflurane on Aδ and C reflexes remained unchanged during observations lasting more than 3 h.) The effect of an equilibrated concentration of 1.5% sevoflurane on Aδ and C sympathetic reflexes evoked by stimulation of the tibial nerve was observed, stability of the preparation confirmed for 20 min, and new “control” data obtained showing the depressant effect of 1.5% sevoflurane on Aδ and C sympathetic reflexes remained unchanged during observations lasting more than 3 h.)

At the end of each experiment, naloxone (2 mg i.v.) was administered to reverse the effects of fentanyl; return of the reflexes to control levels indicated that during the experiment there had been no damage to the spinal cord and no deterioration in recording. Finally i.t. injection of 2 ml plain lignocaine (2%) rapidly abolished not only the tibial nerve reflexes but also efferent spontaneous sympathetic activity, indicating that the spread of the potential effect of the injections of fentanyl encompassed both afferent fibres in the tibial nerve and the efferent sympathetic fibres from which recordings were made.

**ANALYSIS OF DATA**

All data are expressed as mean (sd). Statistical analysis was performed by analysis of variance (ANOVA) followed, when there were significant changes, by paired $t$ tests. Differences were considered statistically significant when the $P$ was $<0.05$. A regression analysis was used to calculate the slope and 95% confidence intervals of the fentanyl (i.t.) dose–response curves using a computer program Statview + Graphic (Macintosh, Abacus Concepts, Berkeley, CA, USA); the ED$_{25,50,75}$ values, together with the 95% confidence intervals for fentanyl’s depressive effects, were calculated from the dose-response curves.
Sevoflurane, fentanyl and somatosympathetic reflexes

Results

Figures 2 and 3 show examples of typical recordings of spontaneous sympathetic activity and Aδ- and C-fibre-mediated somatosympathetic reflexes, evoked by supramaximal electrical stimulation of the tibial nerves, from two preparations. They show that whereas sevoflurane causes a major increase in the effect of i.t. fentanyl on somatosympathetic reflexes, there was no measurable effect on spontaneous sympathetic activity attributable to fentanyl and sevoflurane alone or in combination.

SOMATOSYMPATHETIC REFLEXES: SEVOFLURANE

The mean ED50s with 95% confidence intervals were 3.8 (3.5–4.4) % and 2.7 (2.4–3.0) % for Aδ and C reflexes respectively. These values are slightly higher than those for Aδ (3.7%) and C reflexes (2.6%) evoked by radial nerve stimulation, which could be because of the larger size of the tibial nerve and the different site of access of the nerve fibres to the spinal cord.

SOMATOSYMPATHETIC REFLEXES: FENTANYL

Both C- and Aδ-evoked responses were depressed by fentanyl in a dose-dependent manner. It can be seen in figs 2 and 4A that fentanyl has a greater effect on C reflexes compared with Aδ and the ratio of the mean doses required to cause the same percentage reduction in C and Aδ responses was approximately 1:2.5. However at a total dose of 128 μg the C reflexes were completely abolished while the mean value of Aδ responses was reduced to 10% of control, with complete abolition in two of five preparations. Naloxone (2 mg i.v.) reversed the effects of fentanyl on both C and Aδ reflexes. Its ED50 calculated from the dose response curves, for Aδ reflexes was

Mean Aδ and C evoked responses were depressed to 84.5% (P<0.05) and 72.5% (P<0.001) of baseline by 1.5% sevoflurane (figs 3 and 4B). This confirms the results of a previous study, which showed that the differentially greater effect of sevoflurane on C reflexes compared with Aδ reflexes was comparable to that of μ opioids.17

Effect of 1.5% sevoflurane

Figure 3  Recordings of the effects of fentanyl administered intrathecally, during the administration of 1.5% sevoflurane, on evoked responses (ER) and spontaneous sympathetic activity (RSNA) in renal nerves. ER: lower traces = average transient of 16 responses; upper traces = rectified integral of the averaged signals. RSNA: lower traces = transient amplified signals; upper traces = rectified and integrated signals. 1 = control; 2 = 20 min after 1.5 % sevoflurane administration; 3–5 = recordings at total cumulative doses of 16, 32 and 64 μg fentanyl i.t. combined with continued sevoflurane administration (1.5%); 6 = 30 min after sevoflurane withdrawal; 7 = 5 min after naloxone (2 mg i.v.).
10.5 (8.7–16.2), 35.6 (23.4–46.5) and 120.7 (95.8–135.8) μg and for C reflexes they were 4.3 (2.1–6.5), 14.2 (11.3–16.4) and 46.5 (35.7–54.3) μg respectively.

COMBINATION OF 1.5% SEVOFLURANE AND FENTANYL

The effect of sevoflurane was to cause a major increase in the effectiveness of fentanyl such that C and A<sub>a</sub> responses were completely abolished in all preparations by mean total doses of 32 μg and 64 μg of fentanyl respectively; A<sub>a</sub> and C-fibre mediated somatosympathetic reflexes evoked by supramaximal electrical stimuli (30 V intensity, 0.5 ms duration, 0.33 Hz frequency) of tibial nerves. Results are means expressed as percentage of control values (SD). Comparison with control: *P < 0.05; **P < 0.001; ***P < 0.001.

SPONTANEOUS SYMPATHETIC ACTIVITY

There were no significant changes in spontaneous sympathetic activity in any preparation throughout the present study, that is, with i.t. fentanyl, which abolished both A<sub>a</sub> and C reflexes, with sevoflurane alone in ED<sub>50</sub> concentration for A<sub>a</sub> and C reflexes, and i.t. fentanyl used to abolish A<sub>a</sub> and C reflexes during the administration of 1.5% sevoflurane (figs 2 and 3).

RESTING HEART RATE AND MEAN ARTERIAL PRESSURE

Fentanyl

There were no significant changes in heart rate or MAP throughout the study.

Combination of 1.5% sevoflurane and fentanyl

Administration of 1.5% sevoflurane caused a decrease in mean heart rate from 168 beats min<sup>−1</sup> (bpm) to 144 bpm (ns), after which there was no further reduction during the administration of fentanyl. The mean MAP was reduced from 120 mm Hg, that is and 4.8 μg respectively instead of the observed values of 8.5 and 3.7 μg; also the predicted/observed dose ratios for the A<sub>a</sub> and C reflexes were 2.1 and 1.3 respectively. These observations indicate marked synergism between the two drugs, which is supported by the isobolograms shown in fig. 5.
the control level, to 98 mmHg (P < 0.01) by 1.5% sevoflurane without any further reduction during the administration of i.t. fentanyl. Both mean heart rate and MAP returned to within control values 30 min after sevoflurane withdrawal, that is before the administration of naloxone (2 mg i.v.).

Discussion
This study shows for the first time that the combined antinociceptive effects of an inhalation anaesthetic agent and a spinally administered analgesic drug are not merely additive but highly interactive. It reveals the full extent of the synergistic interaction between the antinociceptive effects of sevoflurane and intrathecally administered fentanyl. It also confirms that sevoflurane, like fentanyl, has a greater effect on C compared with A6 reflexes.

The observation of the greater sensitivity of C-fibre responses to opioids is in keeping with previous work on morphine, fentanyl and alfentanil administered intrathecally,18–20 which also applies to fentanyl, alfentanil, sufentanil and morphine when administered intravenously.16,21–23 The ratio of the doses of i.t. fentanyl that depress A6 and C reflexes, that is, 2.5:1, is of a similar order of magnitude to that for fentanyl during i.v. administration.16

Previous studies have shown that a reduction of the MAC of volatile agents is caused by opioids administered intrathecally or extradurally in both rats and humans.11–14 For example, Drasner and colleagues reported that 0.75 mg morphine injected intrathecally in rats produced a 40% reduction in the MAC of halothane,11 and more recent work has indicated that the extradural administration of morphine (4 mg) decreased the MAC of halothane by 28% in humans.11 It has also been reported that the MAC of halothane is reduced in a dose-related manner by fentanyl administered extradurally in man.15 The present study shows not only that sevoflurane causes a large augmentation of the effect of intrathecal fentanyl on Aδ- and C-fibre-mediated somatosympathetic reflexes but also that their interaction is highly synergistic.

Opioid receptors are found in the dorsal horn of the spinal cord on the terminals of small-diameter primary afferent fibres,3,4 and it is well known that opioids, when administered intrathecally, depress nociceptive reflexes because of their actions in the dorsal horn area.12,14 It has been suggested that in the spinal cord of the rat, which is the animal model most often used in experimental pain studies, 70% of the opioid receptor population are type μ, 24% are δ receptors and 6% are κ.26 However, in the spinal cord of humans κ receptors are the predominant type at 50%, followed by μ, with 40%, with relatively fewer δ receptors.27 The dog model used in the present study has a spinal cord length of comparable scale to that of the human, with an opioid-receptor distribution also similar to that in humans,28 and is therefore a better model for clinically relevant experimental studies.

Interaction between the brain and spinal cord with respect to the action of inhalation agents has been the subject of a limited amount of work, summarized by Eger and colleagues.10 The concentration required to produce amnesia and unconsciousness is approximately 25–40% of that needed to suppress purposeful movement.6 Rampil and colleagues reported that in rats, decerebration does not decrease the anaesthetic concentrations required to produce immobility.7 It has also been shown that if only supraspinal structures are exposed to inhalation anaesthetic agents, the concentrations required to produce anaesthesia and reflex depression are more than double those required in the normal situation when the spinal cord is also involved.8 It would appear that the spinal cord is a principal site of action for inhalational anaesthetic agents.

Synergy between the actions of drugs depends on interactive pharmacodynamic effects, and these will clearly be influenced temporally by pharmacokinetics and spatially by functional interactions at different anatomic sites.20 However, hitherto, there are no published reports on the type of pharmacodynamic interaction that occurs between an analgesic drug and an inhalation anaesthetic agent, such as fentanyl and sevoflurane.

At cellular level it has been demonstrated that opioid μ agonists increase potassium conductance in presynaptic neuronal membranes, causing membrane hyperpolarization and reduced excitability.30 Opioids may reduce neuronal transmission by both inhibition of postsynaptic cell firing and through presynaptic reduction of neurotransmitter release.31 Volatile anaesthetic agents reduce voltage-operated calcium currents in the central nervous system so as to cause a reduction in neurotransmitter release at presynaptic terminals.32 It has also been shown that sevoflurane activates postsynaptic GABA_A receptors in CA1 pyramidal neurons of rat hippocampus.33 Therefore, sevoflurane and fentanyl may activate neurotransmitter systems, which may couple the same second-messenger system or G proteins34 and therefore provide a basis for their synergistic interaction. However, for a single anaesthetic agent, the output of neuronal networks, modified by changes in the function of the cells within them, may show interactive amplification of effect to cause the anaesthetic state. The combined effects of anaesthetic and analgesic drugs will also reflect mechanisms fundamental to that state and any unified hypothesis of anaesthesia must embody the phenomenon of synergism.

Fentanyl, injected intrathecally in the lumbar region, depresses somatosympathetic responses evoked by stimulation of tibial nerves and recorded in renal nerves; this action occurs with no effect on spontaneous efferent sympathetic activity or responses to radial nerve stimulation, indicating that fentanyl’s effects are localized to the afferent pathway.19 This is true for other μ opioids unless there is significant systemic absorption, when there are effects on all afferent pathways and higher centres that regulate sympathetic activity.20 For sevoflurane, it has been shown that compensatory baroreflexes, although depressed, retain sufficient activity at concentrations up to 3–4% to prevent effects on either spontaneous efferent sympathetic pathways or the heart rate.35 Hence neither fentanyl nor sevoflurane would be expected to cause a reduction in spontaneous sympathetic activity, and this was confirmed for both drugs when administered alone and in combination throughout this study.

In conclusion, it has been shown that intrathecally administered fentanyl and inhaled sevoflurane...
interact with a high level of synergism to depress somatosympathetic reflexes. The dorsal horns of the spinal cord are likely to be a principal site for their interaction. In the concentration and doses used, either alone or in combination, they did not modify spontaneous sympathetic activity.

References


