Suppression of parasympathetic reflex vasodilatation in the lower lip of the cat by isoflurane, propofol, ketamine and pentobarbital: implications for mechanisms underlying the production of anaesthesia

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**Summary**

We have compared the effects of isoflurane, propofol, ketamine and pentobarbitone on parasympathetic reflex vasodilatation to investigate their involvement in GABA-mediated synaptic inhibition, enhancement of which is thought to underlie the action of many anaesthetic agents. In cats anaesthetized with urethane–α-chloralose, parasympathetic reflex vasodilatation in the ipsilateral lower lip was elicited by electrical stimulation of the central cut end of the lingual nerve. Isoflurane and pentobarbitone both produced marked dose-dependent inhibition of this vasodilator response. In contrast, propofol and ketamine had no effect on parasympathetic reflex vasodilatation. Administration of a GABA antagonist (picrotoxin) reversed the inhibition produced by isoflurane (previous results) and pentobarbitone (present study). Our results suggest that isoflurane and pentobarbitone inhibit parasympathetic reflex vasodilatation via a GABA-mediated mechanism, but that propofol and ketamine have no such effect. Our results with propofol cast doubt on its presumed mechanism of action as an anaesthetic. (Br. J. Anaesth. 1998; 81: 563–568).

Keywords: anaesthetics volatile, isoflurane; anaesthetics i.v., propofol; anaesthetics i.v., ketamine; hypnotics barbiturate, pentobarbital; parasympathetic nervous system; brain, GABA

The precise mechanism by which many anaesthetic agents exert their action remains unknown, as does their site of action.1–3 However, there is a considerable body of evidence to suggest that enhancement of GABA-mediated synaptic inhibition may be a common mode of action among anaesthetic agents.4–7 In particular, barbiturates,6–12 volatile anaesthetics (isoflurane,11,13,14 halothane,13,15 enflurane,1,13,14 sevoflurane9) and propofol have been reported to enhance the effect of GABA within the central nervous system.17–20 Indeed, the main sites at which these anaesthetics act to induce anaesthesia are considered to be GABA receptors. In contrast, ketamine is an anaesthetic which is considered to have little effect on the action of GABA.6,11,17,21,22

We have reported previously that at clinically used concentrations, isoflurane produced marked suppression of parasympathetic reflex vasodilatation in the orofacial area in cats anaesthetized with urethane–α-chloralose.23 Pretreatment with the GABA receptor antagonist picrotoxin significantly attenuated isoflurane-induced inhibition, suggesting that GABAergic neurones are activated by isoflurane and then inhibit parasympathetic neurones23 within the central nervous system, possibly by a direct action at the brainstem level. This may suggest that inhalation anaesthetics act on the parasympathetic reflex pathway by a mechanism similar to that by which they induce anaesthesia. However, in our preliminary experiments with propofol, which is considered to exert its action via enhancement of GABA, we detected no inhibitory effect on parasympathetic reflex vasodilatation. These results question whether anaesthetics considered to enhance GABA within the central nervous system inhibit the parasympathetic reflex response also.

In this study, we have compared the effects of pentobarbitone, propofol, isoflurane and ketamine on parasympathetic reflex vasodilatation and examined the involvement of GABA receptors in the modulation of this response.

**Materials and methods**

The studies were reviewed by the Committee on the Ethics of Animal Experiments, Tohoku University School of Medicine, and the study was carried out in accordance with the Guidelines for Animal Experiments issued by Tohoku University School of Medicine, and The Law (No. 105) and Notification (No. 6) of the Japanese Government. We studied 27 adult cats of both sexes, weighing 2·2–3·7 kg, sedated initially with ketamine 30 mg kg⁻¹ i.m. and anaesthetized with a mixture of urethane 100 mg kg⁻¹ i.v. and α-chloralose 50 mg kg⁻¹ i.v., which was used to produce basal anaesthesia unless otherwise noted. In experiments to test the effect of urethane combined with α-chloralose on parasympathetic reflex vasodilatation, propofol 9 mg kg⁻¹ h⁻¹ i.v. was used as the basal anaesthetic. The effects of anaesthetics on parasympathetic reflex vasodilatation were tested 2–3 h after the initial sedation had been produced with ketamine. As the effects of ketamine are short-lasting (30–60 min after i.m. injection), the initial sedative dose should not have affected subsequent responses. Basal anaesthetics were supplemented when neces-

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In the anesthetized animals, the trachea was intubated, and neuromuscular block was produced by pancuronium (Mioblock, Organon) 0.4 mg kg\(^{-1}\) i.v. initially, supplemented with 0.2 mg kg\(^{-1}\) h\(^{-1}\). The lungs were ventilated artificially via the tracheal cannula using a mixture of 50% air–50% oxygen. End-tidal concentration of carbon dioxide and inspired and end-tidal concentrations of the inhalation anaesthetics were monitored continuously using an infrared analyser (Capnomac Ultima, Datex Co. Helsinki, Finland). The ventilator was set to maintain end-tidal concentration of carbon dioxide at 4.9 kPa. Systemic arterial pressure and heart rate were recorded directly from a femoral artery catheter connected to a Statham pressure transducer. Ringer’s solution was infused continuously at a rate of approximately 8 ml h\(^{-1}\). Rectal temperature was maintained at 37–38°C using a heating pad.

In all experiments, the vagus and sympathetic trunks in the neck were cut bilaterally before stimulation. This avoided involvement of the cervical sympathetic in any reflex effects, and ensured that only non-vagal parasympathetic effects were involved in the study.

To elicit parasympathetic reflex vasodilatation in the lower lip, the central cut end of the lingual nerve (LN) was stimulated electrically at a supramaximal intensity (30 V) with 2 ms pulses at 10 Hz for 20 s. The lingual nerve was stimulated where indicated by the filled circles at times shown on the figure. The action of anaesthetics on parasympathetic reflex vasodilatation. Electrical stimulation was applied to the central cut end of the lingual nerve (LN). The blood flow response of lower lip blood vessels was measured by a laser Doppler flowmeter (LDF). NTS = Nucleus of the solitary tract. ISN = Inferior salivatory nucleus.

![Diagram](Image)

Figure 1: Schematic representation of the putative peripheral and central pathways and mechanisms involved in the action of anaesthetics on parasympathetic reflex vasodilatation. Electrical stimulation was applied to the central cut end of the lingual nerve (LN). The blood flow response of lip blood vessels was measured by a laser Doppler flowmeter (LDF). NTS = Nucleus of the solitary tract. ISN = Inferior salivatory nucleus.

**Results**

Figure 2 shows typical examples of the effects of propofol 9 mg kg\(^{-1}\) h\(^{-1}\) and 1.6% isoflurane on the

![Graph](Image)

Figure 2: Typical examples of the effects of propofol 9 mg kg\(^{-1}\) h\(^{-1}\) and 1.6% isoflurane on the increase in lower lip blood flow (LBF) elicited by electrical stimulation of the central cut end of the lingual nerve in vago-sympathectomized cats. The lingual nerve was stimulated where indicated by the filled circles at supramaximal intensity (30 V) with 2 ms pulses at 10 Hz for 20 s. Top: effect of propofol; bottom: effect of isoflurane. The double-headed arrow indicates the period during which each anaesthetic was administered (30 min). “Back control” indicates the response to lingual nerve stimulation 20 min after cessation of administration of anaesthetic.
increase in ipsilateral blood flow in the lower lip elicited by electrical stimulation of the central cut end of the lingual nerve. Administration of propofol had little effect on the vasodilator response during the period of study, but isoflurane produced significant inhibition during inhalation, followed by complete recovery within 20 min of cessation of isoflurane.

Figure 3 shows the effects of propofol 9 mg kg\(^{-1}\) h\(^{-1}\), ketamine 10 mg kg\(^{-1}\), pentobarbital 20 mg kg\(^{-1}\) and 1.6% isoflurane on parasympathetic reflex vasodilatation elicited by stimulation of the central cut end of the lingual nerve. Propofol and ketamine had little or no effect on parasympathetic reflex vasodilation at any dose (2.25, 4.5 and 9 mg kg\(^{-1}\) h\(^{-1}\)) produced reductions of 7.0 (1.6) % (n = 5, ns), 7.5 (4.0) % (n = 5, ns) and 4.0 (1.7) % (n = 7, ns), respectively.

Figure 6 shows the time-related effect on parasympathetic reflex vasodilatation at any dose (2.25, 4.5 and 9 mg kg\(^{-1}\) h\(^{-1}\)) produced reductions of 7.0 (1.6) % (n = 5, ns), 7.5 (4.0) % (n = 5, ns) and 4.0 (1.7) % (n = 7, ns), respectively.

In the experiments illustrated in figure 6, propofol
The presence of propofol and each is given as mean (SEM). The increase in lower lip blood flow (LBF) elicited by lingual nerve stimulation in vago-sympathectomized cats. Vasodilator responses to electrical stimulation of the central cut end of the lingual nerve (30 V, 10 Hz, 2 ms, 20 s) in vago-sympathectomized cats. Vasodilator responses are expressed as a percentage of the control response (evoked in the presence of propofol) and each is given as mean (SEM). The number of cats used was four. Open and shaded columns show, respectively, the increase in lower lip blood flow elicited by lingual nerve stimulation in the absence (control; propofol only) and presence of urethane–α-chloralose. Filled column shows the increase in lower lip blood flow evoked by lingual nerve stimulation 20 min after cessation of propofol infusion (i.e. during urethane–α-chloralose alone).

9 mg kg⁻¹ h⁻¹ was used to produce basal anaesthesia. There were no significant differences between the control response (or back control) and responses evoked in the 30–120 min after administration of urethane–α-chloralose alone.

**Discussion**

We have shown that parasympathetic reflex vasodilation in the lower lip of the cat was suppressed by both pentobarbital and isoflurane (but not by propofol or ketamine), and that pentobarbital-induced suppression was largely reversed by picrotoxin, a GABAₐ antagonist. We have postulated previously on the basis that i.v. administration of a GABA antagonist such as bicuculline elicits a parasympathetically mediated response similar to the reflex response. Furthermore, we have reported recently that inhalation anaesthetics such as isoflurane, sevoflurane and halothane (each at 1 MAC) markedly inhibited parasympathetic reflex vasodilation in the lower lip and palate that was elicited by electrical stimulation of the central cut end of the lingual nerve in vaso-sympathectomized cats anaesthetized with urethane–α-chloralose, and that prior administration of picrotoxin, a GABA antagonist, reversed this inhibitory effect of isoflurane. These results suggest that inhalation anaesthetics produce inhibition of parasympathetic reflex responses via activation of GABA receptors or increase in GABA, or both (fig. 1). As the inhibitory action of isoflurane was unaffected by decerebration, it is apparently exerted within the brainstem.  

Our finding that administration of picrotoxin reversed the inhibition of parasympathetic reflex vasodilation produced by pentobarbital (fig. 4) is in agreement with our previous results using isoflurane. It is well known that the rostral gustatory zone of the nucleus of the solitary tract (NST) of the rat and the caudal viscerceptive NST contain both GABA and the principle enzyme involved in the degradation of GABA, GABA transaminase. Further, the preganglionic parasympathetic cell bodies of the superior and inferior salivatory nuclei receive direct projection from the NST, in addition to one from the parabrachial nucleus. Our data are consistent with the idea that both isoflurane and pentobarbital activate GABA receptors (and/or increase GABA release) within the NST, and that this leads to inhibition of parasympathetic reflex vasodilation (fig. 1). However, at present this idea is speculative.

Ketamine is thought to inhibit N-methyl-D-aspartate (NMDA) receptors and have little effect on GABA receptors within the central nervous system. Our data (fig. 3) showed that administration of ketamine had no effect on parasympathetic reflex vasodilation, suggesting that NMDA has no influence on this type of reflex vasodilation.

Propofol has been used widely in clinical practice, and also in experimental studies both in vivo and in vitro. It has been reported to enhance the action of GABA within the central nervous system, for example in the cerebral cortex, hippocampus and spinal dorsal horn. However, no inhibitory effect of propofol on parasympathetic reflex vasodilator response was observed in our cats anaesthetized with urethane–α-chloralose (fig. 2). These results question whether GABA-enhancing anaesthetics necessarily produce inhibition of parasympathetic reflex vasodilation. Nevertheless, except in the case of propofol, there seemed to be a close relationship between supposed GABA-enhancing effect and inhibition of parasympathetic reflex vasodilation. Interestingly, there is evidence of differential effects among anaesthetics, in that propofol has been reported to bind to GABA receptors other than those affected by pentobarbital and isoflurane. There are at least two explanations for the inability of propofol to inhibit parasympathetic reflex vasodilation: (i) propofol has no ability to enhance the action of GABA within the brainstem, even though it has such an ability in other parts of the CNS. (ii) Propofol cannot produce a further reduction in the parasympathetic reflex vasodilator response recorded under basal urethane–α-chloralose anaesthesia because urethane–α-chloralose had already inhibited the relevant GABA receptors. The first possibility seems unlikely as it requires propofol to enhance the action of GABA at several sites within the brain and spinal cord, but not within the brainstem pathway mediating parasympathetic reflex vasodilatation. However, we cannot completely exclude this possibility. The second possibility (that urethane–α-chloralose strongly inhibits the relevant GABA receptors) is more likely because urethane has minimal effects on circulatory dynamics, and α-chloralose has comparatively few depressant effects on central nervous structures. Moreover, our experiments showed that urethane–α-chloralose had
no effect on parasympathetic reflex vasodilatation when propofol was used as the basal anaesthetic (fig. 6).

Propofol has been reported to decrease sympathetic activity more than parasympathetic activity, and to produce bradycardia, including asystole. Our findings that propofol had no effect on parasympathetic reflex vasodilatation when propofol was used as the basal anaesthetic (fig. 6).

References

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