Propofol anaesthesia in mice is potentiated by muscimol and reversed by bicuculline

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We have examined the role of γ-aminobutyric acid (GABA) neurones in propofol anaesthesia in mice using the righting reflex. Propofol i.p. increased the percentage of loss of the righting reflex in a dose-dependent manner with an ED50 value of 140 (95% confidence limits 123–160) mg kg–1 (n=40; eight animals per dose, five doses per dose–response curve). The ED50 for propofol decreased significantly to 66 (58–75) mg kg–1 in the presence of the GABA A receptor agonist muscimol 1 mg kg–1 i.p. (n=40) (P<0.05). In contrast, the ED50 increased significantly to 240 (211–274) mg kg–1 in the presence of the antagonist bicuculline 5 mg kg–1 i.p. (n=40) (P<0.05). Our results suggest that propofol anaesthesia may be mediated, at least in part, by GABA neurones.

Keywords: anaesthetics i.v., propofol; brain, GABA; pharmacology, muscimol; pharmacology, bicuculline; mouse; receptors, amino acid

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In the mammalian central nervous system (CNS), γ-aminobutyric acid (GABA) functions as an inhibitory neurotransmitter. General anaesthetics enhance inhibitory synaptic transmission mediated by GABA. Thus GABAergic neurones are potential targets for general anaesthetic action.1 Propofol has also been shown to enhance GABAergic neurotransmission in biochemical, electrophysiologic and molecular biological studies.1–3 However, no study has investigated the underlying mechanism of propofol anaesthesia using a behavioural model. In this in vivo study, we have examined the ability of a GABA A receptor agonist to potentiate propofol anaesthesia and an antagonist to reverse anaesthesia in mice.

Methods and results

The study was approved by the Committee of Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine. We studied adult male ddY mice, weighing 34–40 g (n=120). Each animal was used only once, and only one study per animal was conducted. All drugs were administered i.p. The GABA A receptor agonist muscimol was administered 30 min before administration of propofol 56, 65, 74, 86 or 100 mg kg–1 (n=40; eight animals per dose), as 30 min is required to reach the peak anticonvulsant effect and this effect persists for 1–1.5 h.4 The GABA A receptor antagonist bicuculline was given simultaneously with propofol 150, 175, 200, 230 or 260 mg kg–1 (n=40; eight animals per dose), because we confirmed in preliminary studies that bicuculline-induced convulsions occur within 5 min after injection and that the peak effect of propofol anaesthesia occurs 6 min after administration. The control group was pretreated with saline or vehicle solution and then injected with propofol 115, 130, 150, 175 or 200 mg kg–1 (n=40; eight animals per dose). Specific (muscimol–propofol or bicuculline–propofol) and control (saline–propofol or vehicle–propofol, respectively) treatments were performed on the same experimental day.

Mice were examined individually in a glass beaker (13.5 cm diameter × 19 cm high). After administration of propofol, we tilted the beaker by hand to an angle of approximately 45° with a horizontal plane in triplicate at each recording time. Righting reflex was assessed and recorded every 2 min after injection for 30 min by a blinded observer. No righting within 10 s in all three studies was considered ‘absence of the righting reflex’. Righting within 10 s at least once in three studies was considered ‘presence of the reflex’.

The number of animals who lost the righting reflex out of the total that received a specific treatment was used to calculate the percentage loss of righting reflex. The 50% effective dose (ED50) for loss of righting reflex, 95% confidence limits of that value and the significance of
The GABA_A receptor antagonist bicuculline reversed potentiated propofol-induced anaesthesia in mice. In contrast, the GABA_A receptor agonist muscimol, at an approximate ED_{50} value (4.8 \pm 3.9–5.9) mg kg^{-1} for inducing tonic–clonic seizures in a preliminary study, shifted the propofol dose–response curve to the right (Fig. 1). The ED_{50} for propofol increased significantly from 140 (123–160) to 240 (211–274) mg kg^{-1} in the presence of bicuculline (P<0.05).

Comment

We have shown that the GABA_A receptor agonist muscimol potentiated propofol-induced anaesthesia in mice. In contrast, the GABA_A receptor antagonist bicuculline reversed propofol anaesthesia (Fig. 1). In addition, it has been reported that propofol inhibits bicuculline-induced tonic–clonic seizures in mice. These behavioural findings suggest that propofol augments GABAergic neuronal responses.

In our study, the anaesthetic dose that abolished the righting reflex in 50% of animals was used to measure anaesthetic potency; that is, it represents an anaesthetic ED_{50}. Probably the best estimate of anaesthetic potency is the minimum alveolar (or anaesthetic) concentration (MAC) of an agent that produces immobility in 50% of those subjects exposed to a noxious stimulus, such as a tail clamp. However, it has been shown that the anaesthetic ED_{50} in the mouse, as determined by loss of the righting reflex, correlates closely with MAC in humans. Thus the anaesthetic ED_{50} appears to reflect MAC and to be relevant as a model for induction of clinical anaesthesia.

The potent and specific GABA_A receptor agonist muscimol was administered systemically in this study. However, there are no detailed bioavailability or pharmacokinetic data for the CNS after administration of muscimol i.p. It has been reported that in vitro, muscimol is 27 times more potent than 4,5,6,7-tetrahydroisoxazolo-[5,4-c]-pyridin-3-ol (THIP), another GABA_A receptor agonist, in inhibiting [3H]GABA binding to rat cerebellar membranes. In contrast, after systemic administration, muscimol is only three times more potent than THIP in inhibiting reticulata cell firing, possibly because muscimol passes the blood–brain barrier less readily. However, despite its slightly poorer penetration of the blood–brain barrier, muscimol is the most potent anticonvulsant drug among 16 GABAmimetic drugs, including THIP, even after i.p. administration. Muscimol produces a peak anticonvulsant effect within 0.5–1 h after i.p. injection and its action persists for 1–1.5 h. These findings indicate that systemic administration of muscimol may be an effective way to examine its action on the CNS.

Systemic administration of muscimol induces depressant actions, such as impairment of motor co-ordination and sedation, especially in high doses. There is a possibility, therefore, that synergy between muscimol and propofol may occur via action at different sites. Indeed, this type of synergy is an acknowledged phenomenon (e.g. \alpha_2-adrnergic receptor agonists and general anaesthetics). Moreover, bicuculline may antagonize the depressant action of propofol, regardless of its site of action, because bicuculline is a potent neuroexcitant and convulsant. These possibilities suggest that propofol may produce anaesthesia independent of GABA neurones. In fact, propofol adheres to the correlation between anaesthetic potency and lipid solubility exhibited by other general anaesthetics (it obeys the Meyer–Overton rule) and its action is pressure-reversible. Thus interpretation of our behavioural data should be made with caution.

In contrast with the evidence of a non-specific action of propofol, most studies of the underlying mechanism of propofol anaesthesia have focused on GABAergic neurones.
It has been shown in biochemical studies that propofol produces a marked increase in the affinity of $[^3H]$GABA binding to rat cortical membrane preparations, and potentiates muscimol-induced stimulation of $^{36}$Cl$^-$ uptake in membrane vesicle preparations.\(^2\) Electrophysiological and molecular biological studies have also revealed that propofol enhances GABA-induced Cl$^-$ currents elicited on GABA$\_A$ receptors expressed by Xenopus oocytes.\(^3\) These in vitro findings suggest that postsynaptic GABA$\_A$ receptors are important sites for propofol action. In addition, it has been reported that propofol increases spontaneous $[^3H]$GABA release, and potentiates high potassium-evoked $[^3H]$GABA release from rat cerebrocortical synaptosomes.\(^9\) Propofol has also been shown to inhibit synaptosomal uptake of $[^3H]$GABA in rat striatum.\(^10\) Thus considerable evidence has shown that propofol exerts both pre- and postsynaptic actions on GABAergic neurones, but the bulk of the evidence supports the latter.

In summary, both previous in vitro findings and our behavioural data suggest that propofol anaesthesia is mediated, at least in part, by GABAergic neurones.

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