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Effects of propofol on guinea pig respiratory smooth muscle

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Abstract: The effects of propofol on the tone of guinea pig respiratory smooth muscle was studied both in vitro and in vivo. In vitro, the activity of propofol on tracheal smooth muscle was investigated using a force displacement transducer for isometric tension responses. Isoproterenol was used as the control. Concentration-response curves to propofol and isoproterenol were obtained using a cumulative dose schedule. Propofol (0.32–10.24 $\mu g \cdot ml^{-1}$) relaxed the tracheal smooth muscle in a concentration-dependent manner, but was less potent than isoproterenol (equipotent molar ratio 29 000:1). This effect of propofol was not affected by prior administration of atropine, propranolol, prazocin, or yohimbine, and it did not appear to be mediated via calcium antagonism. The solvent for propofol (10% intralipid) had no effect on the tracheal smooth muscle in vitro. The in vivo study measured the effect of propofol on lung pressure in deeply anesthetized guinea pigs using histamine induced bronchoconstriction. Propofol (1–4.5 $mg \cdot kg^{-1}$, i.v.) exhibited neither relaxant nor constrictor effects. It is possible that the effects of propofol observed in vitro are due to nonspecific action, while the finding of no effect in vivo could be due to different tissue sensitivity to propofol, i.e., tracheal smooth muscle may be more responsive than bronchial smooth muscle. Propofol does not seem to have any deleterious effects on airway smooth muscle.

Key words: Anesthetics, Intravenous, Propofol, Bronchoconstriction, Smooth muscle relaxation

Introduction

Anesthetic drugs may affect airway smooth muscle tone. Halogenated inhalational agents like halothane are known to cause bronchodilation and inhibit bronchoconstriction [1]. Propofol (2,6-di-isopropyl phenol) is a short-acting intravenous anesthetic that is widely used for the induction and maintenance of anesthesia, in day-case surgery and also for sedation of critically ill patients in the intensive care unit [2]. Propofol has a profound (central) depressant effect on respiration, often blunting the ventilatory response to an increase in inspired CO_2 [3]. Less well known is the direct effect of propofol on the airway. Gigarini et al. [4] reported that propofol antagonizes fentanyl-induced bronchoconstriction, while Pedersen [5] found that propofol inhibited bronchospasm in two patients with hyperactive airway disease.

It is fairly well established that propofol causes an endothelium-independent vasodilator effect through direct action on vascular smooth muscle [6–8], presumably by increasing sequestration of Ca^{2+} in intracellular organelles [9–10]. We have been interested in the effects of propofol on airway smooth muscle, and therefore its potential for use in patients who are prone to bronchoconstriction (asthmatics). A report by other workers [9] showed that propofol caused relaxation of guinea pig trachea smooth muscle tone induced by various agents. In the present study, the effects of propofol on guinea pig airway smooth muscle were investigated both in vivo and in vitro (resting/spontaneous tone) and an attempt made to examine the mechanism of action for the observed effects. A preliminary account of this report was presented in abstract form [11].

Materials and methods

Animals

Adult guinea pigs of either sex weighing 250–400 g were used in accordance with our institutional guidelines on animal experimentation. The animals were obtained from the Laboratory Animal Centre (Singapore) and kept in air conditioned rooms (21° ± 2°C; humidity 70%–80%) in the Animal Holding Unit (N.U.S) for at

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least 48 h before the experiments. Food and water were supplied ad libitum. Each animal was used only once.

Drugs

Propofol (Diprivan, ICI, Cheshire, UK) was used as supplied (i.e., 1% emulsion of 10% soya bean oil, 2.25% glycerol, and 1.2% purified egg phosphatide in water). Isoproterenol, propranolol, atropine, prazocin, and yohimbine were dissolved in physiological saline solution to the requisite concentrations. The vehicle for propofol was 10% Intralipid (Kabi Vitrum Inc., Almeda, CA, USA). Krebs-Ringer, Ca-free Krebs-Ringer, and KCl (30 mM) solutions were also used.

In vitro preparation of guinea pig tracheal smooth muscle

Tracheal smooth muscle was prepared by a modification of the method of Akcasu [12]. The guinea pigs were killed by stunning and exsanguination. The trachea from the larynx to the carina was rapidly removed and placed in Kreb's physiological solution. The composition the Krebs-Ringer solution was (mM): NaCl-117, KCl-4.8, $MgSO_4$-1.2, KH_2PO_4-1.2, $NaHCO_3$-25, $CaCl_2$-2.5, and glucose-5.7. Adhering tissue was removed by blunt dissection after which the trachea was cut into single rings. Seven tracheal rings were tied together with the circular muscle running on the same side of the chain, placed into a 25-ml organ bath containing Krebs-Ringer solution (20 ml) and connected to a force-displacement transducer for the measurement of isometric tension. The organ bath was kept at 37°C and gassed with 95% O_2 and 5% CO_2 mixture. The tissue was suspended under a force of 0.3 g and left to equilibrate for at least an hour before the start of the experiment. Changes were monitored on a Hewlett Packard recorder (Model 7702 B).

Concentration-response curves were constructed for propofol using a cumulative dose schedule. Isoproterenol was used as a positive control for the relaxant effect. Average contact times were 7 and 4 min for propofol and isoproterenol respectively, using a dosing cycle of 25–30 min. To ensure that any observed effects were attributable to propofol, the vehicle (intralipid) was tested separately using the protocol for propofol.

In an attempt to elucidate the mechanism(s) involved, we added propranolol (7.7×10^{-6}M), atropine (6.8×10^{-6}M), prazocin (5.2×10^{-6}M), or yohimbine (5.6×10^{-6}M) 1 min before propofol (1.3×10^{-5}M) or isoproterenol (3.7×10^{-6}M). The various concentrations were determined in a preliminary study. The role of Ca^{2+} was studied using Ca^{2+}-free Krebs-Ringer solution (prepared as for Krebs-Ringer except that $CaCl_2$ was excluded) followed by depolarization with 30 mM KCl as described in detail by Raeburn et al. [13]. After 20 min, Ca^{2+} 0.1 or 0.5 mM was added; these doses were adopted from those used by earlier workers [9] and were found to be effective. When the response to exogenous Ca^{2+} had leveled off, the tissues were washed with Ca^{2+}-free Krebs-Ringer solution, incubated for a further 60 min and then depolarized with KCl as above. After 5 min in the presence of KCl, propofol (6.7×10^{-6}M or 1.3×10^{-5}M) was added and left to equilibrate for 15 min, after which Ca^{2+} was added. Responses obtained in the presence of propofol are expressed as a percentage of the maximum pretreatment control values.

In vivo lung pressure in anesthetized guinea pigs

We used a modification of the method described by James [14] for measuring lung pressure in deeply anesthetized animals. Four animals were anesthetized using pentobarbital 30 $mg \cdot kg^{-1}$ i.p. with additional 7.5 $\mu g \cdot kg^{-1}$ at 10-min intervals until deep surgical anesthesia was achieved—slow, deep abdominal breathing and no reaction to painful stimulus of the extremities. The animal was ventilated using room air. A cut was made in the lower half of the trachea and a Y-ventilation cannula inserted therein. One arm of the cannula was connected to a constant volume respiratory pump (Ugo Basile # 7025, Camerio-Va, Italy); the other was connected back to the pump in a closed circle system. A side-arm of the outflow was connected to a pressure transducer (Ugo Basile # 7020). The pump setting was 70 strokes per min with a stroke volume of 2–3 ml varied according to the weight of the animal. Changes in airway pressure were recorded on a two-channel recorder (UgdBasile # 7070).

The jugular vein was cannulated and connected to a three-way tap for drug administration, while the carotid artery was cannulated for simultaneous recording of blood pressure. Drugs were administered at 7- to 10-min intervals and flushed with 0.4 ml of saline. A 60-W lamp was placed above the tracheal tube to prevent condensation. Minimum lengths of connecting tubes were used in order to reduce dead space. Bolus injections of histamine were used to induce bronchoconstriction, and the bronchodilator effect of the drug(s) was inferred from a reduction in lung pressure. After two constant consecutive responses to the same dose of histamine were obtained, isoproterenol or propofol was administered 1 min before the control dose of histamine. The control dose of histamine was then repeated until the response returned to basal levels. When two consecutive doses of propofol gave similar responses, the next dose was given.

Data analysis

Relaxant responses to propofol and isoproterenol in vitro are expressed as a percentage of spontaneous tone. Concentration-relaxation curves were calculated by linear regression analysis and computer fitting. Where applicable, differences between parameters were tested by the unpaired Student's *t*-test. Relaxant effects in vivo are expressed as a reduction in histamine-induced lung pressure.

Results

Effects of isoproterenol and propofol on guinea pig trachea smooth muscle

Both isoproterenol and propofol had a relaxant effect on guinea pig trachea smooth muscle. Typical concentration-response tracings are shown in Fig. 1. The relaxant effect of isoproterenol was greater than that for propofol. The respective EC_{50} values obtained from the

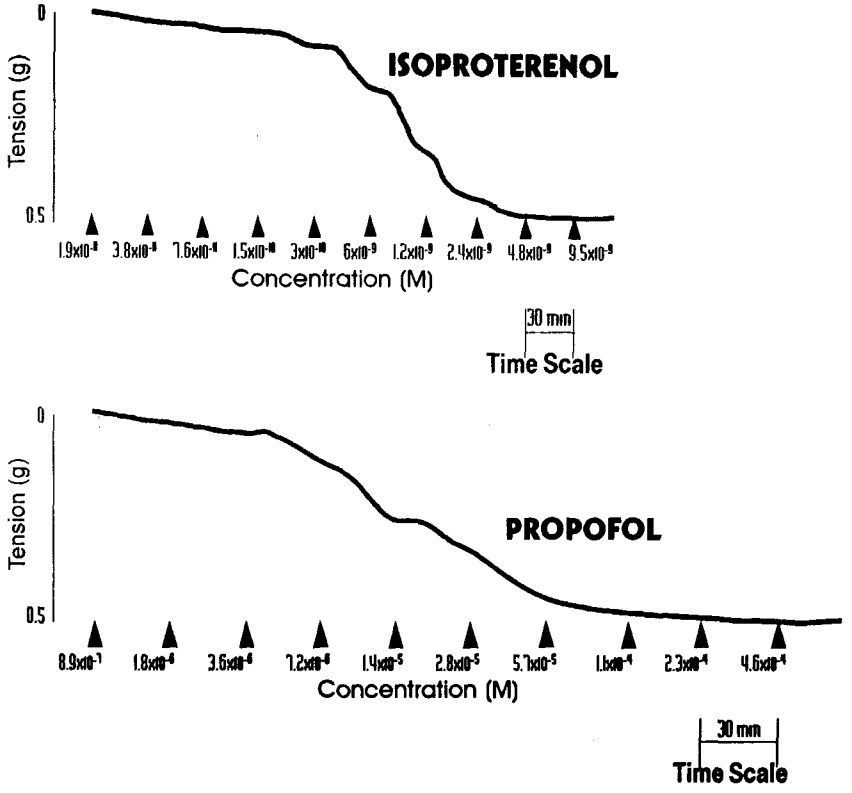

Fig. 1. Typical cumulative concentration-response profile of guinea pig tracheal smooth muscle to isoproterenol and propofol. The concentration for isoproterenol is in the 10^{-11} to 10^{-9}M range, and that for propofol 10^{-7} to 10^{-4}M. Equipotent ratio of isoproterenol: propofol is 1:29 000

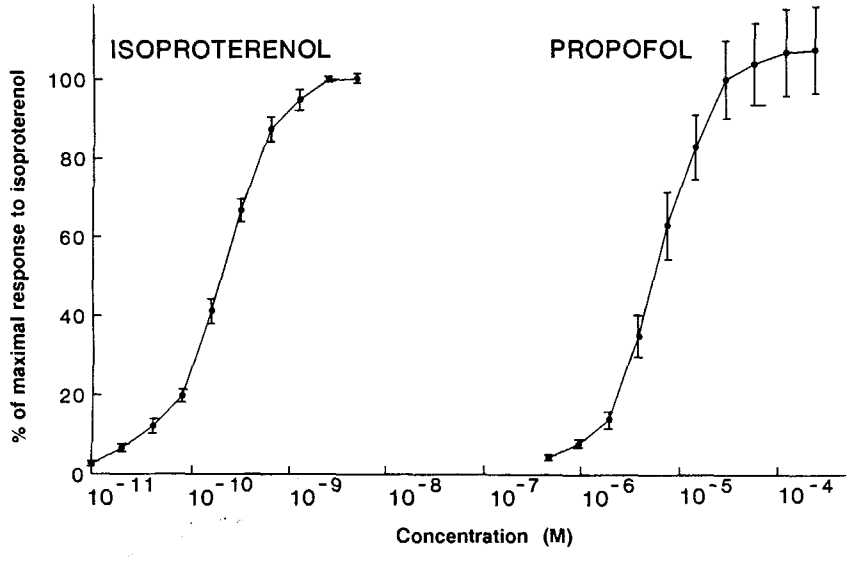

Fig. 2. Concentration-response curves for isoproterenol and propofol. Responses are standardized as a percentage of maximal effect of isoproterenol (at 1.9×10^{-7} M). Propofol seems to give >100% response because of this reference point. Data points are mean ± SE, $n = 10$

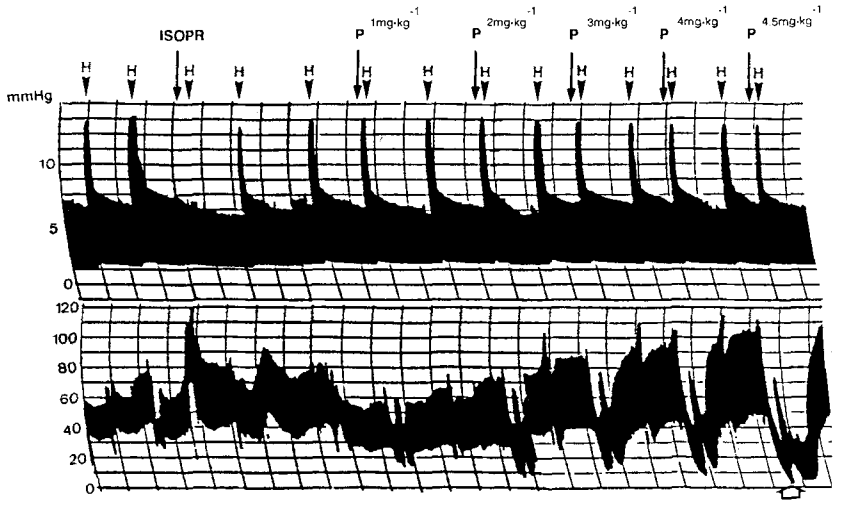

Fig. 3. Changes in guinea pig lung pressure in response to histamine (*H*), isoproterenol (*ISOPR*) or propofol (*P*), *upper panel*. Histamine 5 μg·kg^{-1}, i.v. produced bronchoconstriction (rise in lung pressure) which was blocked by pretreatment with ISOPR but not P. *Lower panel* shows corresponding changes in blood pressure; note that propofol at 4.5 mg·kg^{-1} produces profound hypotension (*arrow*)

concentration-response curve were 2.25 × 10^{-10}M and 6.52 × 10^{-6}M for isoproterenol and propofol (the equipotent molar ratio being 1:29 000). Figure 2 is the concentration-response curve showing a clear right shift of the response curve from isoproterenol to propofol. The vehicle for propofol (10% intralipid) had no effect on the tone of the tracheal smooth muscle. Propranolol abolished the relaxant effect of isoproterenol but not that of propofol; atropine, prazocin, and yohimbine had no effect on the activity of isoproterenol or propofol. Propofol did not affect the contractile response to Ca^{2+} in KCl depolarized guinea pig trachea smooth muscle.

Effects of isoproterenol and propofol on histamine-induced bronchoconstriction

Histamine (5 μg·kg^{-1}, i.v.) produced a typical submaximal bronchoconstriction (Fig. 3). This effect was attenuated by prior administration of isoproterenol (1.2 μg·kg^{-1}, i.v.) but not by intravenous administration of propofol (1.0–4.5 mg·kg^{-1}). Typical responses are shown in Fig. 3. Propofol doses of 5 mg·kg^{-1} or higher were invariably fatal. Propofol alone did not show any bronchoconstrictive effect, nor did it potentiate histamine-induced bronchoconstriction.

Discussion

The data presented show that propofol relaxes guinea pig trachea smooth muscle under basal (spontaneous) tone in vitro. This is in line with reports based on stimulated tone [9]. However, administration of up to 4.5 mg·kg^{-1}, i.v. of propofol had no effect on lung pressure in the anesthetized animals. The findings for isoproterenol are consistent with its relaxant effects through β-adrenoceptor activation; to this extent, therefore, it was a useful control. The guinea pig was chosen for this study because it exhibits spontaneous resting airway tone—a characteristic shared by humans [15]—and in vitro guinea pig tracheal smooth muscle shows marked pharmacological similarity to human tracheal smooth muscle [16]. Therefore, findings based on guinea pig airway smooth muscle may have relevance to clinical practice.

The relaxant effect of propofol on guinea pig trachea smooth muscle observed in vitro was concentration dependent, and apparently was not due to the vehicle as evidenced by the lack of effect of 10% intralipid. The levels of propofol achieved in the organ baths using the protocol in this study (0.32–10.24 μg·ml^{-1}) are comparable to those in human plasma during propofol anesthesia (effective range 2.5–10 μg·ml^{-1}) [17]. However, this is of limited interpretive value since the in vivo and in vitro situations are quite different, and it is not clear how plasma concentrations relate to the levels achieved in the tissues. Moreover, the propofol preparation we used is only slightly water soluble, and may therefore have low tissue contact in the organ bath. This premise has been investigated by others [9] and it was found that a water-based preparation of propofol gave better results in vitro than did the oil-based one. Propofol was 30 000 times weaker than isoproterenol, and its effects were nonadrenergic (β_2-activation is relaxant whereas the role of α-mechanisms is thought to be insignificant), noncholinergic (muscarinic receptor activation leads to contraction, while inhibition leads to relaxation), and not due to calcium antagonist activity. Taken together, these findings suggest that the relaxant effect of propofol observed in vitro may be a nonspecific depressant one.

An attempt was made to investigate the possible involvement of the cyclooxygenase pathway using indomethacin, but even at low concentations (<1 μg·ml^{-1}) indomethacin abolished the resting tone

of the tissue and therefore could not be used to investigate the effects on spontaneous tone. Some studies have used indomethacin to achieve reproducible and consistent contractile responses in the isolated guinea pig trachea [9,18], but these were targeted at stimulated tracheal tone. Since propofol increases the binding of calcium to isolated mitochondria, increased sequestration of Ca^{2+} in intracellular organelles is a possible mechanism for the smooth muscle relaxant effect of propofol [9–10]. In any case, we were unable to demonstrate any effect of Ca^{2+} on propofol activity.

In vivo, propofol had no apparent effect on histamine-induced bronchoconstriction. It has previously been reported that propofol 2.5 $mg \cdot kg^{-1}$, i.v. had no effect on resting bronchomotor tone in anesthetized guinea pigs [19]. Our findings show that even in the presence of bronchoconstriction, propofol has no effect on bronchomotor tone. It is noteworthy that propofol did not induce bronchoconstriction or aggravate histamine—induced bronchoconstriction; this could cautiously be extrapolated to mean that it is safe to use propofol even in patients predisposed to bronchoconstriction. The apparent discrepancy between the in vivo (none) and in vitro (relaxant) effects of propofol may be partly due to the fact that different targets were assessed. In vitro, changes in tracheal smooth muscle tone were sought, whereas in vivo it was changes in bronchomotor tone. Responses of the tracheal smooth muscle may differ from those of the bronchial smooth muscle [20–21]. Furthermore, in vivo (plasma) concentrations of propofol decline rapidly after a bolus injection due to redistribution and extensive metabolism [22], whereas in vitro the levels change only a small degree, if at all. It is also possible that the complex interaction of factors maintaining basal bronchomotor tone (neural/humoral) in vivo [23] could obscure a weak bronchodilating effect of propofol. The lack of an effect on lung pressure seems to be at variance with clinical reports of a bronchodilator effect of propofol [4–5]. It is unlikely that the difference is due to doses used since even subanesthetic doses were beneficial in the clinical case reports. There may be a difference in sensitivity to propofol between the human and guinea pig lower airway, notwithstanding their close pharmacological similarity alluded to above.

In conclusion, propofol at concentrations similar to those achieved in plasma in clinical practice [16] caused a weak, albeit reproducible, relaxation of guinea pig tracheal smooth muscle. This effect was apparently nonspecific. In vivo, however, propofol had no relaxant or constrictor effects on bronchial smooth muscle. Based on these data, it may be concluded that the respiratory depressant effects of propofol are not enhanced by local action of the drug on the airway itself.

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