European Journal of Pharmacology 765 (2015) 463–471

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Propofol protects against opioid-induced hyperresponsiveness of airway smooth muscle in a horse model of target-controlled infusion anaesthesia ☆

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ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form
3 September 2015
Accepted 4 September 2015
Available online 11 September 2015

Keywords:
Bronchial hyperresponsiveness
Equine isolated bronchi
Propofol
Remifentanil
Drug interaction
Veterinary anaesthesia

Chemical compounds studied in this article:
Propofol
Remifentanil

ABSTRACT

General anaesthesia in horses is associated with elevated mortality rate in subjects suffering of heaves. Target-controlled infusion (TCI) of sedative–hypnotic medications and opioids represents a total intravenous anaesthesia (TIVA) method validated in veterinary medicine. Since there are no data concerning the impact of these classes of drugs in inducing bronchial hyperresponsiveness (BHR) in horses, the aim of this study was to investigate the effect propofol and remifentanil on the contractile response of equine airway smooth muscle.

The influence of propofol and remifentanil on the contractile response of equine isolated bronchi to electrical field stimulation (EFS) was assessed. The role of capsaicin-sensitive sensory nerves, inducible nitric oxide synthase (iNOS) and neurokinin 2 (NK_2) receptor was also assessed. The interaction analysis was performed by Bliss Independence theory. Experiments were repeated in desensitized and passively sensitized airways.

Remifentanil induced BHR in both non-sensitized and passively sensitized bronchi, ($+56.33 \pm 8.01\%$ and $+99.10 \pm 14.52\%$, respectively; $P < 0.01$ vs. control) and propofol significantly prevented this effect ($P > 0.05$ vs. remifentanil). The inactivation of capsaicin-sensitive sensory nerves *via* desensitization and blocking NK_2 receptor inhibited the BHR remifentanil-induced ($P > 0.05$ vs. controls). The inhibition of iNOS reverted the protective effect of propofol on the BHR induced by remifentanil (non-sensitized: $+47.11 \pm 7.70\%$; passively sensitized: $+70.51 \pm 11.39\%$; $P < 0.05$ vs. control). Propofol synergistically interacted (overall $\approx 40\%$) in preventing the remifentanil-induced BHR.

Remifentanil induces BHR *via* stimulating capsaicin-sensitive sensory nerves that facilitate the cholinergic neurotransmission through the activation of NK_2 receptor. The propofol/remifentanil combination may be safely administered in course of TCI–TIVA procedures also in heaves affected horses.

1. Introduction

General anaesthesia is associated with elevated mortality rate (0.6–1.8%) in healthy horses and may reach the 5% in ill equines (Johnston et al., 2002). These data are impressive if compared with both humans ($< 0.01\%$) (Lagasse, 2002) and small animals ($\approx 0.1\%$) values (Dyson et al., 1998).

Respiratory complications represent an important cause of post-operative death in horses (Johnston et al., 2002; Johnston et al., 1995). Hypoventilation and consequent hypoxaemia are common problems during anaesthesia (William et al., 2007) and pulmonary dysfunction occurs more often in horses than in other

Abbreviations: ANOVA, analysis of variance; BHR, bronchial hyperresponsiveness; *E*, effect; EFS, electrical field stimulation; EC_{50}, concentration inducing 50% E_{max}; E_{max}, maximal effect; iNOS, inducible nitric oxide synthase; NKA, neurokinin A; NK_n receptor, neurokinin n receptor; RAO, recurrent airway obstruction; S.E.M., standard error of the mean; TCI, target-controlled infusion; TIVA, total intravenous anaesthesia

☆Recommended Section Assignment: Pulmonary, gastrointestinal and urogenital pharmacology.

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http://dx.doi.org/10.1016/j.ejphar.2015.09.007
0014-2999/

domestic species (Klein, 1990). During anaesthesia the cardiac ejection fraction may be reduced by one-third and up to 30% of pulmonary blood flow may not take part in gas exchange, thus increasing venous admixture and pulmonary arteriovenous shunt (Bidwell et al., 2007). Therefore, sustaining bronchorelaxation and avoiding bronchospasm in equines should be a primary objective for veterinary anaesthetists.

It has been demonstrated that anaesthesia may produce a considerable impairment of lung function (Tiefenthaler et al., 2011) and that even sedative procedures may induce bronchospasm if the drug mixture is not adequately balanced (Rogliani et al., 2013).

Recurrent airway obstruction (RAO) is a common respiratory disorder in horses with heaves (Williamson and Davis, 2007) and bronchial hyperresponsiveness (BHR), that characterize RAO, may affect the perioperative pulmonary function in equines (Seddighi and Doherty, 2012). Although there are no studies concerning the perioperative risk of bronchospasm in horses suffering from heaves, it has been demonstrated that the induction of anaesthesia may cause BHR in patients with normal or pathological airways (Hirshman and Bergman, 1990).

Total intravenous anaesthesia (TIVA) *via* plasma-site concentrations by target-controlled infusion (TCI) has the great advantage to be easy controlled and to have a short recovery time, representing the more diffuse choice for anaesthesia (Auckburally et al., 2008; Bras et al., 2009; Glen, 1998; Musk and Flaherty, 2007; Pei et al., 2014; Pol and Vincent, 2006). The combination of the sedative–hypnotic agent propofol with opioids is a commonplace in human TCI anaesthesia and has been recently validated also in veterinary medicine (Musk and Flaherty, 2007). In veterinary anaesthesia the current tendency is to move from volatile anaesthetics to TCI–TIVA procedures and, thus, novel propofol/opioids combinations have been recently proposed (Gozalo-Marcilla et al., 2015; Knobloch et al., 2006; Seddighi and Doherty, 2012).

The μ-opioid receptor agonist remifentanil and propofol are drugs of choice for TCI anaesthesia (Bryson et al., 1995; Mertens et al., 2003a). The infusion of remifentanil for 60 min did not induce cardiopulmonary side effects in healthy isoflurane-anesthetized horses, and the recovery score was similar to that of control group (Benmansour et al., 2014). Remifentanil was also safely administered for 13 h in a horse to maintain anaesthesia in combination with sevofluorane and dexmedetomidine, suggesting that this opioid may be suitable for long and invasive surgical procedures (Benmansour and Duke-Novakovski, 2013). Propofol administered in combination with medetomidine provided clinically effective anaesthesia in horses, although assisted ventilation should be considered to minimize respiratory depression (Oku et al., 2011). Therefore, since anaesthesia induced and maintained exclusively with propofol may induce hypoventilation and hypoxaemia, adequate preanaesthetic sedation and oxygen supplementation are required in horses anaesthetised with this drug (Muir et al., 2009). In fact cardiovascular parameters remained within acceptable values in artificially ventilated horses anesthetized for 4 h with propofol administered by TIVA (Umar et al., 2007). Finally, although the stroke volume decreases dose-dependently due to decrease of cardiac contractility during anaesthesia with continuous propofol infusion, the findings of available clinical trials suggest that also propofol, similarly to remifentanil, may be suitable for long-term TIVA in horses undergoing a range of surgical procedures (Bettschart-Wolfensberger et al., 2005; Oku et al., 2006).

Nevertheless, although conflicting data exist on the role of these medications in inducing BHR (Brown et al., 2001; Joshi et al., 2002; Koo and Eikermann, 2011; Nishiyama and Hanaoka, 2001; Rogliani et al., 2013), recently some of us have demonstrated that remifentanil may produce bronchospasm even at sedative concentrations, at the least in human (Rogliani et al., 2013). Therefore, we have hypothesized that remifentanil can induce BHR also in horses, especially at higher concentrations used for deep anaesthesia, and that propofol may play a protective role against the opioid-induced BHR *via* an indirect influence on the cholinergic neurotransmission in equine.

Thus, since there are no studies concerning the influence of propofol and remifentanil on equine airway smooth muscle tone, the aim of this study was to investigate the effect of these medications on the equine BHR in an *ex vivo* model of TCI anaesthesia.

2. Materials and methods

2.1. Ethics considerations

This study has been carried out in accordance with directive 2010/63/EU revising directive 86/609/EEC concerning the protection of animals used for scientific purposes. In order to not specifically sacrifice animals for research aims, the tissues used in this study have been obtained from equine routinely killed in an slaughterhouse for food, agreeing with the second criterion proposed by Russel and Burch on the principles for more ethical use of animals in testing (Russell et al., 1959). This investigation was in agreement with the guidelines from the local Veterinary Board and carried out as a no-profit study.

2.2. Tissue preparation

Equine airways were collected from 21 healthy horses (11 male and 10 female; aged 2.5 ± 0.1 years; weighted kg 362.5 ± 48.4). Samples were placed into Krebs–Henseleit buffer solution (NaCl, 119.0 mmol; KCl, 5.4 mmol; $CaCl_2$, 2.5 mmol; KH_2PO_4 mmol, 1.2 mmol; $MgSO_4$, 1.2 mmol; $NaHCO_3$, 25.0 mmol; glucose, 5.5 mmol; pH 7.4) containing indomethacin (5 μM) and transported to the laboratory. None of the subjects were under pharmacological treatment or showed clinical and anatomopathological signs of respiratory infections.

Tissues were allowed to equilibrate for 90 min during which time the Krebs–Henseleit buffer solution was changed every 10 min. Samples were mounted on hooks where one hook was attached with thread to a stationary rod and the other hook tied with thread to an isometric force displacement transducer. During equilibration, passive tension (2.0–2.5 g) was determined.

The isometric change in tension was measured using a force transducer (Fort 100 WPI, 2Biological Instruments) and the responsiveness was assessed by acetylcholine (100 μM). On reaching a plateau response, rings were washed three times and allowed to stabilize for a further 30 min as described elsewhere (Matera et al., 2005, 2008, 2011b).

2.3. Drugs preparation

The following drugs and compounds were used in this study: acetylcholine (Sigma-Aldrich, Italy), BYK191023 (Santa Cruz Biotechnology), capsaicine (Sigma-Aldrich, Italy), GR159897 (Santa Cruz Biotechnology, US), indomethacin (Sigma-Aldrich, MI, Italy), neurokinin A (NKA) (Sigma-Aldrich, Italy), propofol (AstraZeneca, Italy), remifentanil (GlaxoSmithKline, Italy) and Substance P (Sigma-Aldrich, Italy).

Drug stock solutions were prepared in distilled water, excluding capsaicine and indomethacin, which were dissolved in ethanol, and GR159897 that was dissolved in dimethyl sulfoxide. Indomethacin was after added to Krebs–Henseleit buffer solution. All drugs were then diluted in Krebs–Henseleit buffer solution to be used at the final concentration in the isolated organ baths