Effects of morphine, H-Tyr-D-Arg-Phe-Lys-NH₂ (DALDA) and B-HT920 on non-cholinergic nerve-mediated bronchoconstriction in pithed guinea-pigs

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1 Electrical stimulation (1 ms, 5 Hz, 80 V) for 5 or 15 s at the level of C4–T1 in the spinal canal of artificially respired pithed guinea-pigs (which had received intravenously (i.v.) (+)-tubocurarine chloride 2 mg kg⁻¹, atropine sulphate 2 mg kg⁻¹ and pentolinium tartrate 5 mg kg⁻¹) caused constriction of airways, indicated by increased insufflation pressure.

2 This non-cholinergic constriction was inhibited by morphine (1–3 mg kg⁻¹, i.v.), the peripherally acting μ -receptor agonist, H-Tyr-D-Arg-Phe-Lys-NH₂ (DALDA, 0.1–1 mg kg⁻¹, i.v.) or the α_2 -adrenoceptor agonist B-HT920 (1–3 mg kg⁻¹, i.v.)

3 The effects of either morphine (3 mg kg⁻¹, i.v.) or DALDA (1 mg kg⁻¹, i.v.) were inhibited by naloxone (3 mg kg⁻¹, i.v.). Idazoxan (3 mg kg⁻¹, i.v.) inhibited the anti-constrictor effect of B-HT920 (3 mg kg⁻¹, i.v.), but not that of DALDA (0.1 mg kg⁻¹, i.v.).

4 Thus activation of peripheral μ -opioid receptors or α_2 -adrenoceptors inhibits airways constriction induced by non-cholinergic nerve stimulation in the pithed guinea-pig. This preparation therefore provides a further method for the *in vivo* examination of the effects of drugs on non-cholinergic tracheobronchial constrictor nerve function.

Introduction

Transmural electrical stimulation of guinea-pig isolated bronchi causes both cholinergic and long-lasting non-cholinergic contractions, the latter being possibly due to the release of neuropeptides such as substance P (Andersson & Grundström, 1983). Such non-cholinergic contraction of guinea-pig bronchi is inhibited by μ -opioid agonists such as morphine and [D-Ala², NMePhe⁴, Gly-ol⁵]enkephalin (DAMGO, Frossard & Barnes, 1987), and also by stimulation of α_2 -adrenoceptors (Grundström *et al.*, 1984). *In vivo* opioid receptor- and α_2 -adrenoceptor-mediated inhibition of non-cholinergic guinea-pig airway constriction, induced by electrical stimulation of peripheral vagus nerves has also been demonstrated (Grundström & Andersson, 1985; Belvisi *et al.*, 1988). However, in previous *in vivo* studies of non-cholinergic bronchoconstriction in the guinea-pig, the central nervous system has been intact, thus allowing possible complications arising from central actions of drugs and the effects of anaesthesia.

The aim of the present study was to determine the feasibility of using the pithed guinea-pig to examine specific peripheral effects of drugs on constrictor responses of the airways to non-cholinergic nerve stimulation. Stimulation was at the level of C4–T1, and therefore probably involved either visceral or somatic afferent fibres, as ganglionic, cholinergic muscarinic, and skeletal neuromuscular transmission were inhibited by pentolinium, atropine and (+)-tubocurarine respectively. The usefulness of the preparation has been validated by use of the α_2 -adrenoceptor agonist B-HT920 and the opioid receptor agonists morphine and H-Tyr-D-Arg-Phe-Lys-NH₂ (DALDA). DALDA is a recently synthesized μ -receptor selective polar dermorphin analogue which acts peripherally (Schiller *et al.*, 1989a,b).

Methods

Male Dunkin-Hartley guinea-pigs (250–710 g) were anaesthetized with 4% halothane in nitrous oxide (11 min⁻¹) and

oxygen (11 min⁻¹). In each, the left carotid artery was tied and an identifying ligature looped around the right carotid artery. The trachea was cannulated. With the guinea-pig prone, a 2 cm midline incision was made through the skin of the scalp, and a 2 mm hole made with a punch in the sagittal fissure between the orbits. A pithing rod (with an inbuilt electrode at the tip, a modification to that described for use in the rat by Gillespie *et al.*, 1970) and similar to that used by Armstrong & Boura (1973) was inserted via the hole through the foramen magnum and down the full length of the spinal canal.

The tracheal cannula was connected to a ventilator (Ugo Basile 7025) and the animal ventilated with air (10 ml kg⁻¹; 60 strokes min⁻¹). Insufflation pressure was recorded (Grass polygraph model 7D) via a pressure transducer (Gould Statham P23) connected to a side arm of the tracheal cannula. This was used as an index of tracheobronchial resistance. The right carotid artery was cannulated for monitoring blood pressure via a pressure transducer (Gould Statham P23) connected to the polygraph recorder. Triggering of a rate meter by the pulse pressure enabled heart rate to be recorded.

When surgery was completed the rod was withdrawn to leave a length of 4.5–7.0 cm between the electrodes and the point of insertion into the skull. The electrodes then lay at the level of C4–T1. For intravenous administration of drugs a jugular vein was cannulated. Temperature was maintained at 35.5–37°C by placing the animal on a heated table, with radiant heat provided by an overhead lamp.

(+)-Tubocurarine chloride (2 mg kg⁻¹) and atropine sulphate (2 mg kg⁻¹) were administered intravenously to prevent stimulation of nicotinic receptors on skeletal muscle and to block muscarinic receptors respectively. Pentolinium tartrate (5 mg kg⁻¹) was also given intravenously to block nicotinic receptor-mediated ganglionic transmission and possible release of catecholamines from the adrenal medulla.

The cord was stimulated by the electrodes with pulses of 1 ms at 5 Hz and 80 V (Grass stimulator S88). These parameters were chosen to stimulate nerves at a supramaximal voltage to produce a submaximal response from which recovery readily occurred. In initial experiments stimulation was for 5 or 15 s duration, stimulation being repeated at 10 min intervals for a total time of 100 min. In further experiments, when effects on opioid receptors and α_2 -adrenoceptors

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were examined, only one duration of stimulation (15 s) was used, and the test period shortened to 1 h.

Statistical analysis was performed by Student's paired *t* test. Level of significance was taken as $P < 0.05$.

Drugs

Drugs were obtained as follows: (+)-tubocurarine chloride, atropine sulphate monohydrate (Koch Light Laboratories); pentolinium tartrate (May & Baker Ltd.); morphine hydrochloride, naloxone hydrochloride (Sigma); B-HT920 dihydrochloride (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-*d*]azepine) (Boehringer Ingelheim); idazoxan hydrochloride (Reckitt & Colman); DALDA was synthesized as described previously (Schiller *et al.*, 1989b). All drugs were dissolved in saline (0.9 w/v) before use. Doses are given in terms of the salt used.

Results

Electrical stimulation causing non-cholinergic airway constriction

The basal insufflation pressure 2 min before electrical stimulation was 15.5 ± 0.3 mmHg ($n = 30$), and remained constant until drug administration. Electrical stimulation at the level of C4-T1 (5 Hz, 80 V, 1 ms for 5 or 15 s duration alternately) increased the insufflation pressure. The increase in insufflation pressure is shown in Figure 2 and was significantly greater with 15 s stimulation compared with 5 s stimulation ($P < 0.05$). The duration of the constriction (3–5 min) considerably outlasted the period of cord stimulation (Figure 1). Stimulation of a higher frequency (10 Hz) for 15 s caused a greater and more long-lasting response than 5 Hz for 15 s, from which recovery did not occur for at least 10 min (data not shown). Therefore, in all further experiments stimulation was at 5 Hz. Intravenous administration of saline (4 ml kg^{-1} , 0.9% w/v) had no effect on responses which were maintained during repeated stimulation at 10 min intervals over a period of 100 min (Figure 2).

Effect of morphine on non-cholinergic airway constriction

As shown in Figure 3a, morphine (1 mg kg^{-1} , i.v.) significantly attenuated responses to the 5 s electrical stimuli for at least

Figure 2 Non-cholinergic nerve-mediated increase in insufflation pressure to electrical stimulation (● 5 s; ○ 15 s) before and after saline administration (4 ml kg^{-1} , i.v. 0.9% w/v). Vertical lines indicate s.e.mean ($n = 5-7$).

100 min. However, with 15 s periods of stimulation, significant attenuation after this dose was maintained for less than 30 min. After a higher dose (3 mg kg^{-1}) in a further group of animals, responses to 15 s electrical stimuli were significantly attenuated ($P < 0.05$) for at least 60 min (Figure 3b). The inhibitory effect of the latter dose of morphine on 15 s stimuli was antagonized by the μ -receptor antagonist naloxone (3 mg kg^{-1} , i.v., a dose chosen to antagonize completely the effect of morphine), administered 15 min after morphine. Neither morphine (3 mg kg^{-1} , $n = 12$) nor naloxone (3 mg kg^{-1} , $n = 4$) had any significant effect on basal insufflation pressure measured 2 min after intravenous administration (data not shown).

Effect of DALDA on non-cholinergic airway constriction

DALDA (0.1 or 1.0 mg kg^{-1} , i.v.) significantly attenuated ($P < 0.05$) non-cholinergic airway constriction (Figure 4) to 15 s electrical stimulation for at least 60 min. This effect of 1 mg kg^{-1} DALDA was inhibited by naloxone (1 mg kg^{-1} , i.v.). Neither DALDA (1 mg kg^{-1} , $n = 9$) nor naloxone (1 mg kg^{-1} , $n = 5$) had any significant effect on basal insufflation pressure measured 2 min after intravenous administration (data not shown). Idazoxan (3 mg kg^{-1}) an α_2 -adrenoceptor antagonist did not affect attenuation of non-cholinergic

Figure 1 Non-cholinergic nerve-mediated increase in insufflation pressure to electrical stimulation, indicated by the horizontal bars in upper and lower traces, at the level of C4-T1 (5 Hz, 80 V, 1 ms for 15 s) in pithed guinea-pigs. The lower trace has been run on an expanded time scale and the period of stimulation is indicated by the horizontal bar, illustrating both latency and prolongation of the response. Heart rate (HR), blood pressure (BP), and insufflation pressure (IP) are shown.

Figure 3 Non-cholinergic nerve-mediated increase in insufflation pressure to electrical stimulation before and after morphine administration. (a) The effects of morphine (1 mg kg^{-1} , i.v.) on responses to 5 s (●) and 15 s (○) stimuli. Error bars to responses at 5 s are smaller than the symbols. (b) The effect of morphine (3 mg kg^{-1} , i.v.) on responses to 15 s stimuli (○), and its antagonism by naloxone (3 mg kg^{-1} , i.v., ■) administered 15 min later. Vertical lines indicate s.e.mean ($n = 4$). * Significantly different from before morphine, $P < 0.05$; † Significantly different from control responses and those after naloxone, $P < 0.05$.

airway constriction caused by DALDA (0.1 mg kg^{-1} , i.v., $n = 4$, data not shown).

Effect of B-HT920 on non-cholinergic airway constriction

As shown in Figure 5, B-HT920 (1 mg kg^{-1} , i.v.) produced a significant ($P < 0.05$) inhibition of non-cholinergic airway constriction for 30 min, whereas a higher dose (3 mg kg^{-1}), resulted in a significant inhibition ($P < 0.05$) that was maintained for at least 60 min. The α_2 -antagonist idazoxan (3 mg kg^{-1} , i.v.), administered 15 min after B-HT920 (3 mg kg^{-1} , i.v.), effectively inhibited the effect of the latter (Figure 5). Neither B-HT920 ($n = 9$) nor idazoxan ($n = 4$)

Figure 4 Non-cholinergic nerve-mediated increase in insufflation pressure to electrical stimulation before and after H-Tyr-D-Arg-Phe-Lys-NH₂ (DALDA) (○) 0.1 mg kg^{-1} , i.v. and (Δ) 1 mg kg^{-1} , i.v. The effect of naloxone (▲) 1 mg kg^{-1} , i.v. administered 15 min after 1 mg kg^{-1} DALDA is also shown, ($n = 4-5$). * Significantly different from before DALDA, $P < 0.05$. † Significantly different from control response and those also after naloxone, $P < 0.05$. Vertical lines indicate s.e.mean.

Figure 5 Non-cholinergic nerve-mediated increase in insufflation pressure to electrical stimulation before and after B-HT920 administration (○, 1 mg kg^{-1} , i.v.; Δ, 3 mg kg^{-1} , i.v.). Effect of idazoxan (▲, 3 mg kg^{-1} , i.v.) administered 15 min after 3 mg kg^{-1} B-HT920. Vertical lines indicate s.e.mean ($n = 4-5$). * Significantly different from before B-HT920, $P < 0.05$; † Significant difference between control responses and those after idazoxan, $P < 0.05$.

(both 3 mg kg^{-1} i.v.) had any significant effect on the basal insufflation pressure, measured 5 min after intravenous administration (data not shown). However, B-HT920 in contrast to the other agonists did cause a transient rise of basal insufflation pressure, which was significantly raised 2 min after injection ($17 \pm 0.7 \text{ mmHg}$, $P < 0.05$, compared with $15.2 \pm 0.6 \text{ mmHg}$, 2 min prior to drug administration, $n = 9$).

Discussion

Many previous studies of the effects of drugs on neuronal non-cholinergic airways constriction have utilized isolated tissues (e.g. Grundström *et al.*, 1984; Karlsson *et al.*, 1984; Frossard & Barnes, 1987), and some have been performed in anaesthetized guinea-pigs, in which vagal afferent nerves were stimulated in retrograde fashion (e.g. Grundström & Andersson, 1985; Belvisi *et al.*, 1988). The present study demonstrated that non-cholinergic airways constriction can also be elicited by stimulation of the spinal cord in pithed guinea-pigs. Since ganglionic, cholinergic muscarinic, and skeletal neuromuscular transmission were blocked with pentolinium, atropine and (+)-tubocurarine, it is likely that either visceral or somatic afferent nerve fibres were involved in the observed responses. Also, it is unlikely that responses involved liberation of catecholamines from either postganglionic sympathetic efferent fibres or the adrenal medulla, since this would be prevented by pentolinium treatment. The magnitude of the constriction was dependent upon the duration and frequency of stimulation, and was repeatable at 10 min intervals for at least 100 min. The latency of the response *in vivo* has previously been demonstrated by Grundström & Andersson (1985). The airways constriction was inhibited by α_2 -adrenoceptor stimulation with B-HT920, and μ -opioid receptor activation with either morphine or DALDA. The effect of B-HT920 was antagonized by idazoxan, and that of morphine or DALDA by naloxone. These results, therefore, suggest that, as *in vitro* (Grundström *et al.*, 1984; Frossard & Barnes, 1987), non-cholinergic guinea-pig airway constriction can be inhibited by stimulation of either α_2 -adrenoceptors or μ -opioid receptors. However, from the results of the present study it is not possible to be certain that μ -opioid receptors were involved, as δ - and κ -opioid receptor agonists were not used, and naloxone is not totally specific for μ -receptors. The data are analogous to the results obtained by Belvisi *et al.* (1988), who stimulated retrogradely the afferent vagal nerve fibres in the anaesthetized guinea-pig. The site of these inhibitory effects was peripheral, since the spinal cord and brain stem had been destroyed by pithing. Moreover, DALDA is highly polar, and would be unlikely to cause central effects (Schiller *et al.*, 1989b). A likely site of action of the agonists

used in the present study is therefore at, or in the vicinity of, sensory nerve endings within the thoracic cavity. They may possibly be acting to inhibit the release of bronchoconstrictor agents, a release which is in turn mediated by non-cholinergic nerve stimulation.

In summary, results of the present study show that the pithed guinea-pig provides a suitable preparation for the

study of the peripheral effects of drugs on function of the non-cholinergic nerve supply to the tracheo-bronchial tree.

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