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Involvement of central 5-HT_{1A} receptors in the reflex activation of pulmonary vagal motoneurones by inhaled capsaicin in anaesthetized cats

Deborah J. Bootle, *John J. Adcock & [1]Andrew G. Ramage

*Department of Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS and the Academic Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill Street, Hampstead, London, NW3 2PF

1 The aim of the present experiments was to determine whether 5-HT_{1A} receptors play a role in the control of the reflex activation of pulmonary vagal motoneurones. This was carried out by investigating the effects of intracisternal injections (i.c.) of the 5-HT_{1A} receptor ligands, 8-OH-DPAT (50 $\mu g\ kg^{-1}$), buspirone (200 $\mu g\ kg^{-1}$), WAY-100635 (100 $\mu g\ kg^{-1}$), methiothepin (200 $\mu g\ kg^{-1}$) and (−)-pindolol (100 $\mu g\ kg^{-1}$) and the 5-HT_2 receptor antagonist, cinanserin (200 $\mu g\ kg^{-1}$), on the reflex bronchoconstriction evoked by inhaled capsaicin aerosol in α-chloralose anaesthetized, neuromuscularly blocked and artificially ventilated cats. Recordings were made of heart rate, blood pressure and upper tracheal pressure.

2 Central application of all the 5-HT_{1A} receptor antagonists (methiothepin, WAY-100635 and (−)-pindolol) attenuated the reflex bronchoconstriction in the upper trachea. However, the same dose of WAY-100635 given i.v. had no effect on this reflex bronchoconstriction. The 5-HT_{1A} receptor agonist, 8-OH-DPAT (50 $\mu g\ kg^{-1}$) given i.c., potentiated the capsaicin-evoked reflex bronchoconstriction, whereas buspirone (200 $\mu g\ kg^{-1}$) i.c. had no effect. The 5-HT_2 receptor antagonist, cinanserin (200 $\mu g\ kg^{-1}$) also had no effect.

3 It is concluded that the reflex excitation of pulmonary vagal motoneurones by inhaled capsaicin in α-chloralose anaesthetized cats involves the activation of central 5-HT_{1A} receptors.

Keywords: 5-HT_{1A} receptors; pulmonary vagal motoneurones; reflex bronchoconstriction; 8-OH-DPAT; WAY-100635; (−)-pindolol; methiothepin; buspirone; cinanserin, capsaicin aerosol

Introduction

Vagal efferent activity has been demonstrated to be important in maintaining resting airway tone (see Widdicombe *et al.*, 1991) and in mediating reflex bronchoconstrictions (see Coleridge *et al.*, 1989). Further, reflex increases in vagal efferent activity have been implicated in bronchial asthma (see Coleridge *et al.*, 1989; Widdicombe *et al.*, 1991) and increases in vagal tone to the airways have been shown to play an important role in nocturnal asthma (Morrison *et al.*, 1988). The peripheral modulation, by various chemical mediators, of these reflex pathways has been extensively investigated (see Barnes, 1992). However, there is surprisingly little knowledge on the central neuropharmacology of these reflexes, particularly the central neurotransmitter pathways involved in the control of pulmonary vagal motoneurones. It has recently been reported (Anderson *et al.*, 1995) that i.c.v. 5-hydroxytryptamine (5-HT) causes an increase in tracheal pressure in anaesthetized cats implicating a role for central 5-HT receptors in the control of pulmonary vagal motoneurones. In this respect 5-HT_{1A} and 5-HT_2 receptors and 5-HT pathways are well known to play a role in the control of central respiratory drive (Lalley, 1986; King & Holtman, 1990; Shepheard *et al.*, 1991; Sporton *et al.*, 1991; Anderson *et al.*, 1995) and in the control of cardiac vagal motoneurones, particularly 5-HT_{1A} receptors (Ramage & Fozard, 1987; Izzo *et al.*, 1988; 1990; Bogle *et al.*, 1990; Sporton *et al.*, 1991; Chitravanshi & Calaresu, 1992; Futuro-Neto *et al.*, 1993; McCall *et al.*, 1994). Hence the present experiments were carried out to investigate whether 5-HT_{1A} receptors play a role in the control of the reflex activation of pulmonary vagal motoneurones. The particular method chosen to evoke reflex activation of pulmonary vagal motoneurones was that of inhalation of capsaicin, delivered by aerosol, in anaesthetized cats, which activates bronchial and pulmonary C-fibres to produce a bronchoconstriction (see Coleridge *et al.*, 1989). To investigate the role of 5-HT_{1A} receptors in the central modulation of this reflex the non-selective 5-HT_{1A} receptor antagonists, methiothepin and (−)-pindolol (Schoeffter & Hoyer, 1988), the new highly selective 5-HT_{1A} receptor antagonist, WAY-100635 (Forster *et al.*, 1995), the archetypal 5-HT_{1A} receptor agonist, 8-OH-DPAT and the partial agonist, buspirone (Schoeffter & Hoyer, 1988) and finally the 5-HT_2 receptor antagonist cinanserin (see Hoyer & Fozard, 1991) were given i.c. to determine if they would modulate the capsaicin-evoked bronchoconstriction. Preliminary accounts of some of these observations have been given (Bootle *et al.*, 1994; 1995).

Methods

Experiments were carried out on male cats (2.5–4.5 kg). Anaesthesia was induced with halothane (5% in oxygen) and maintained with α-chloralose (80 $mg\ kg^{-1}$, i.v. then 10 $mg\ kg^{-1}\ h^{-1}$). The left femoral artery was cannulated for analsyis of blood gases and pH. Arterial blood pressure was measured with a pressure transducer (Statham P23) and heart rate was derived electronically from the blood pressure signal. The left femoral vein was cannulated for drug administration so that α-chloralose and dimethyl-tubocurarine could be given and the right femoral vein was cannulated for the intravenous administration of drugs. The trachea was cannulated. The animals were artificially ventilated (rate 25 min^{-1}, stroke volume 10 $ml\ kg^{-1}$) with room air by use of a positive pressure pump. Neuromuscular blockade was produced with dimethyltubocurarine (0.125 $mg\ kg^{-1}$ initially, followed 90 min later with 0.0625 $mg\ kg^{-1}\ h^{-1}$). Anaesthesia was assessed by the cardiovascular responses to paw pinch and the pupil size and additional anaesthetic administered if required. Blood samples

[1] Author for correspondence.

were taken from the femoral artery cannula via a 3 way tap and blood gases and pH were monitored with a Radiometer pH/blood gas analyser. Blood gases were maintained betewen 90–130 mmHg Po_2, 35–45 mmHg Pco_2 and pH 7.3–7.4. Adjustments of the respiratory pump volume were made as necessary to maintain blood gas and pH balance. Body temperature was maintained at 36–38°C with a homeothermic blanket system (Harvard). To administer drugs intracisternally (i.c.) the animals were placed in a stereotaxic head holder and the skin and muscles of the neck located at the base of the skull were retracted to expose the atlanto-occipital membrane. The membrane was penetrated with the tip of a 30 gauge needle and drugs were administered in a volume of 10 μl over 20 s.

An air-filled balloon was placed in the trachea above the endotracheal cannula yet below the larynx as described by Adcock (1989). Aerosols were administered to the lower airways only and had no direct contact with the region of trachea in which the tracheal balloon pressure (P_{TB}) was being recorded. Capsaicin aerosols were generated with a modified (Lees & Payne, 1986) DeVilbiss nebuliser placed in the air-intake arm of the ventilation system. Aerosols were delivered as 6 breaths of a 100 μg ml^{-1} solution of capsaicin.

Experimental protocols

The preparation was allowed to stabilize for 30 min. Capsaicin aerosol challenges were given at 30 min intervals throughout and 3 consecutive and comparable capsaicin-evoked responses were obtained on P_{TB} before continuing the experiment, the final of these responses being taken as the 'control' response. Only those animals which showed no sign of tachyphylaxis within these 3 preliminary control responses were used in these experiments. Once the control response had been obtained, the test compound or saline was administered i.v. or i.c. 3 min before the next capsaicin challenge. The response to a final capsaicin challenge was tested at 33 min after the administration of test compound or saline control.

Analysis of results

Baseline values were taken as the mean of 1 min before the addition of drug or vehicle. Changes induced in the capsaicin-evoked response in tracheal balloon pressure, mean blood pressure and heart rate caused by the test drug were compared with a time-matched vehicle control by one-way analysis of variance and were subsequently analysed by Dunnett's test. Changes in baseline levels caused by the drugs were compared with that of the vehicle by Student's unpaired t test. All results are expressed as the mean ± s.e.mean change from baseline values, differences in the mean were taken as significant when $P<0.05$.

Drugs and solutions

The following drugs were used:- 8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH-DPAT), buspirone HCl, cinanserin, methiothepin maleate, (−)-pindolol HCl (Research Biochemicals Inc., Semat Technical Ltd, St. Albans, Herts); N-(2-(4-(2-methoxyphenyl)-l-piperazinyl) ethyl)-*N*-(2-pyridyl) cyclohexanecarboxamide trichloride (WAY-100635; a gift from Wyeth Research U.K., Taplow, Maidenhead, Berks), dimethyl-tubocurarine (Wellcome Research Laboratories, Beckenham, Kent); pentobarbitone sodium (May & Baker Ltd., U.K.); Tween 80, α-chloralose, capsaicin (Sigma Chemical Co., Poole, Dorset). All drugs except capsaicin were dissolved in saline and then pH adjusted if necessary to pH 6.7–7.2 and all doses refer to the salts of the drug. Capsaicin was dissolved in ethanol, Tween 80 and saline in the ratio 1:1:23, a stock solution of 1 mg ml^{-1} prepared and further diluted with saline as required.

Results

Effect of WAY-100635 (100 μg kg^{-1}; i.c. and i.v.), methiothepin (200 μg kg^{-1}; i.c.), (−)-pindolol (100 μg kg^{-1}; i.c.), 8-OH-DPAT (50 μg kg^{-1}; i.c.) buspirone (200 μg kg^{-1}; i.c.) and cinanserin (200 μg kg^{-1}; i.c.) on resting baseline variables

The effect of these drugs on all resting baseline variables are shown in Table 1. WAY-100635 given i.v. and (−)-pindolol, 8-OH-DPAT and buspirone given i.c. caused significant ($P<0.05$) falls in blood pressure. For 8-OH-DPAT and buspirone this fall was also associated with a significant bradycardia. Methiothepin i.c. caused a significant rise in blood pressure.

Effects of drugs on capsaicin-evoked reflex changes

WAY-100635 administered i.c. (a trace from one of these experiments is shown in Figure 1a) when compared with saline (i.c.) significantly inhibited the reflex increase in tracheal balloon pressure (P_{TB}) caused by capsaicin by 50±17% and 55±6% after 3 and 33 min, respectively but not when administered i.v. (Table 2). Methiothepin and (−)-pindolol adminstered i.c. also inhibited the increase in P_{TB} caused by capsaicin. However, 8-OH-DPAT i.c. significantly potentiated the capsaicin-induced reflex increase in P_{TB} by 33±12% after 3 min (Table 2). A trace from one of these experiments is shown in Figure 1b. Buspirone and cinanserin i.c. had no effect on the capsaicin-evoked increase in P_{TB}. The effects of the drugs on the capsaicin evoked reflex bronchoconstriction are summarised graphically in Figure 2.

The bradycardia evoked by capsaicin was unaffected by the

Table 1 Effect of saline and test drugs on resting mean blood pressure (BP, mmHg) and heart rate (HR, beats min^{-1}) and tracheal balloon pressure (P_{TB}; cmH_2O) in α-chloralose anaesthetized cats

Drug (dose; route)	n	*Baseline HR*	Δ *HR*	*Baseline BP*	Δ *BP*	Δ P_{TB}
Saline, 10 μl, i.c.	4	165±17	3±6	104±7	5±2	0.09±0.1
WAY-100635, 100 μg kg^{-1}, i.c.	4	201±17	3±4	94±8	1±2	−0.10±0.1
WAY-100635. 100 μg kg^{-1}, i.v.	4	169±11	−2±3	108±10	−14±3**	−0.12±0.1
Methiothepin, 200 μg kg^{-1}, i.c.	4	149±18	11±12	93±4	18±4*	0.05±0.0
(−)-Pindolol, 100 μg kg^{-1}, i.c.	4	171±5	8±4	97±2	−3±1*	−0.02±0.0
8-OH-DPAT, 50 μg kg^{-1}, i.c.	4	191±9	−24±7*	87±6	−22±11*	−0.01±0.0
Buspirone, 200 μg kg^{-1}, i.c.	4	154±19	−23±8*	101±10	−32±8**	0.92±0.5
Cinanserin, 200 μg kg^{-1}, i.c.	4	179±11	3±2	85±6	2±1	0.08±0.4

Changes (Δ) are compared with saline using a Student's unpaired t test. *$P<0.05$; **$P<0.01$.

test drugs when compared with saline; however, both buspirone and methiothepin significantly reduced the hypotension evoked by inhaled capsaicin (Table 2).

Discussion

The present experiments demonstrate that intracisternal administration of the 5-HT$_{1A}$ receptor antagonist, WAY-100635 (Forster *et al.*, 1995), methiothepin and (−)-pindolol (Schoeffter & Hoyer, 1988) at doses known to inhibit reflex activation of cardiac vagal motoneurones when given i.c. (Bogle *et al.*, 1990) attenuated reflex bronchoconstriction in the upper trachea (P_{TB}) evoked by inhaled capsaicin in anaesthetized, artificially respired and neuromuscular blocked cats. Since capsaicin was not delivered to this part of the trachea (see Methods), the 'bronchoconstriction' observed in this region of the airways must be entirely due to reflex activation of pulmonary vagal motoneurones by capsaicin (see Coleridge *et al.*, 1989). It is possible that the 5-HT$_{1A}$ receptor antagonists are leaking out of the brain and blocking this reflex bronchoconstriction peripherally. However, i.v. administration of the same dose as administered i.c. of the potent and selective 5-HT$_{1A}$ receptor antagonist, WAY-100635 had no effect on the response indicating that the effects of this and the other antagonists, by inference, are acting centrally to attenuate the reflex activation of pulmonary vagal motoneurones. These data, therefore, strongly support the view that 5-HT$_{1A}$ receptors are involved in the activation of pulmonary vagal motoneurones. This is further supported by observations that the archetypal 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT (Schoeffter & Hoyer, 1988) potentiated the reflex bronchoconstriction when applied centrally in the present study. This latter observation strongly supports the view that the effect of

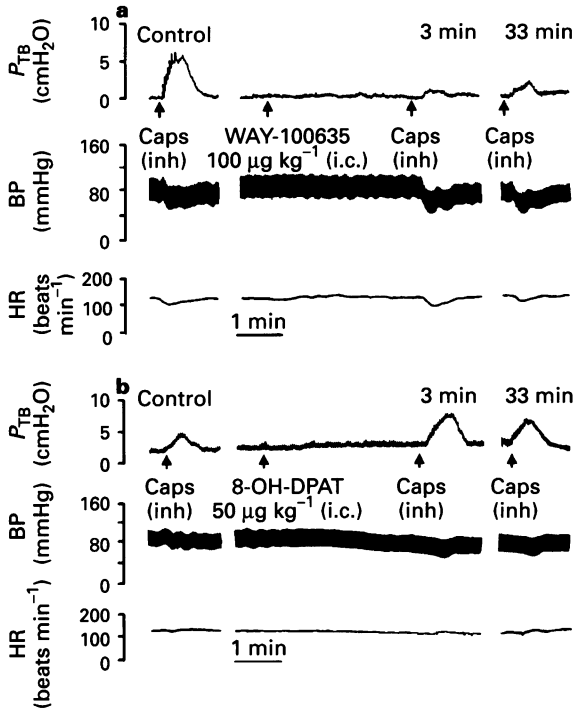

Figure 1 Traces from two separate experiments showing the effect of intracisternal (i.c.) administration of WAY-100635 (a) and 8-OH-DPAT (b) on recordings of tracheal balloon pressure (P_{TB}), arterial blood pressure (BP) and heart rate (HR). The three panels in each section show the effect of inhaled (inh) capsaicin (Caps) delivered to the lower airways on these variables before (Control) and 3 and 33 min after administration of the drugs i.c. in anaesthetized cats.

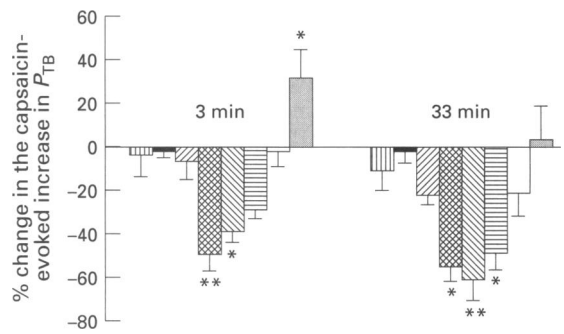

Figure 2 Anaesthetized cats: histograms showing the percentage (%) change in the control response in tracheal balloon pressure (P_{TB}), 'bronchoconstriction', caused by inhaled capsaicin delivered to the lower airways at 3 and 33 min after administration ($n=4$) of (from left to right) (▥) saline 10 μl, i.c.; (■) cinanserin 200 μg kg^{-1}, i.c.; (▨) WAY-100635 100 μg kg^{-1} i.v.; (▩) WAY-100635 100 μg kg^{-1}, i.c.; (▧) methiothepin 200 μg kg^{-1}, i.c.; (▤) (−)-pindolol 100 μg kg^{-1}, i.c.; (□) buspirone 200 μg kg^{-1}, i.c. and (▦) 8-OH-DPAT 50 μg kg^{-1}, i.c.. Each column represents the mean change with s.e.mean. Changes caused by the test substances are compared with that caused by saline by one-way analysis of variance followed by Dunnett's test to compare the means: $*P<0.05$, $**P<0.01$.

Table 2 Effect of test drugs on the hypotension, bradycardia and 'bronchoconstriction' evoked by inhaled capsaicin in α-chloralose anaesthetized cats

Drug (dose, route)	n	*Control* Δ*HR* (beats min^{-1})	*3 min* Δ	*33 min* Δ	*Control* Δ *BP* (mmHg)	*3 min* Δ	*33 min* Δ	*Control* Δ P_{TB} (cmH_2O)	*3 min* %Δ	*33 min* %Δ
Saline, 10 μl i.c.	4	−36±9	−1±3	−2±5	−17±3	3±1	1±3	3.6±1.8	−5±9	−11±9
WAY-100635, 100 μg kg^{-1} i.v.	4	−24±10	4±8	12±20	−19±7	−2±4	−4±6	4.4±2.0	−7±8	−22±4
WAY-100635, 100 μg kg^{-1} i.c.	4	−26±10	−5±6	4±8	−12±7	0±2	10±4	6.3±1.1	−50±7**	−55±6*
Methiothepin, 200 μg kg^{-1} i.c.	4	−22±9	−9±7	−11±6	−27±8	0±4	−23±6**	2.3±0.4	−39±5*	−61±9**
(−)-Pindolol, 100 μg kg^{-1} i.c.	4	−14±4	3±6	14±12	−10±5	0±3	−5±5	7.9±1.1	−29±4	−49±7*
Cinanserin, 200 μg kg^{-1} i.c.	4	−37±17	−10±8	−7±6	−14±5	3±4	−1±2	3.9±0.6	−2±3	−2±5
Buspirone, 200 μg kg^{-1} i.c.	4	−23±3	−1±7	−1±9	−16±4	−11±6*	9±5	3.9±1.9	−2±7	−21±10
8-OH-DPAT, 50 μg kg^{-1} i.c.	4	−29±12	−11±6	−14±8	−4±1	−3±1	−2±2	2.8±1.1	33±12*	4±16

'Control' shows the change (Δ) in resting heart rate (HR), blood pressure (BP) and tracheal balloon pressure (P_{TB}) caused by inhaled capsaicin; 3 and 33 min show the change caused by the test drugs on the control capsaicin-evoked responses. Changes caused by test drug are compared with that caused by saline at 3 and 33 min by one-way ANOVA followed by Dunnett's test. Results are shown as the mean ± s.e.mean. $*P<0.05$; $**P<0.01$.

the above drugs on this reflex bronchoconstriction is not due to a non-specific action. This is further supported by the observations that the two drugs, cinanserin and buspirone, when given i.c., failed to have any effect. The absence of an action of cinanserin is consistent with the view that 5-HT$_2$ receptors are not involved in the reflex activation of vagal motoneurones (Bogle *et al.*, 1990). However, the failure of buspirone, a partial 5-HT$_{1A}$ receptor agonist (Schoeffter & Hoyer, 1988), to have any effect at a dose known to affect reflex activation of cardiac vagal motoneurones in anaesthetized rats (Bogle *et al.*, 1990) and rabbits (Futuro-Neto *et al.*, 1993; Dando *et al.*, 1994a) is surprising. Nevertheless the effect of buspirone does seem to be species-dependant (Dando *et al.*, 1994b) although the effects of buspirone on resting cardiovascular variables in the present experiments are consistent with activation of 5-HT$_{1A}$ receptors (see McCall & Clement, 1994).

Bronchial and pulmonary C-fibre afferents terminate in the nucleus tractus solitarius (Kubin *et al.*, 1991) and preganglionic pulmonary vagal motoneurones are found in the nucleus ambiguus and dorsal vagal nucleus (Kalia, 1981). As well as the nucleus tractus solitarius, projections to preganglionic pulmonary vagal neurones also come from the raphe obscurus and ventral lateral medulla (Haxhiu *et al.*, 1993). In addition, chemical stimulation of the ventral surface increases activity in pulmonary vagal motoneurones (Haxhiu *et al.*, 1987). Further, all these areas are known to contain 5-HT$_{1A}$ binding sites in cats (Dashwood *et al.*, 1988), rats (Thor *et al.*, 1992a, b) and man (Pazos *et al.*, 1987) and 5-HT cell bodies and fibres (Steinbusch, 1981; Calza *et al.*, 1985; Izzo *et al.*, 1988; 1993). Therefore, in the present study, these 5-HT$_{1A}$ receptor ligands could be acting at one or more of the above sites to modulate the reflex activation of pulmonary vagal motoneurones by activation of bronchial and pulmonary C-fibres. It is further possible that as activation of 5-HT$_{1A}$ receptors causes an increased central respiratory drive, and as increases in respiratory drive cause increases in pulmonary vagal motoneurone activity (Mitchell *et al.*, 1984), the ligands may be affecting the activity of these neurones by modulating central respiratory drive. In this respect 8-OH-DPAT has been demonstrated to inhibit the cough reflex in rats (Kamei *et al.*, 1991). Further studies are therefore required before the site/s and precise mechanism/s can be determined by which 5-HT$_{1A}$ receptors and 5-HT pathways influence the activity of pulmonary vagal motoneurones.

Inhaled capsaicin evoked a fall in heart rate and blood pressure, indicative of central sympathoinhibition. The fall in blood pressure was significantly attenuated by buspirone and methiothepin. Again it is difficult to assess whether these drugs interfere directly with this part of the response to inhaled capsaicin, since they caused changes in baseline blood pressure, although other drugs that cause falls in resting blood pressure e.g. 8-OH-DPAT failed to affect the capsaicin-evoked change in blood pressure. Nevertheless, further experiments are needed to investigate the precise mechanism by which buspirone and methiothepin inhibit the capsaicin-evoked fall in blood pressure.

In conclusion these data are the first to demonstrate that, as with cardiac vagal motoneurones, 5-HT$_{1A}$ receptors play an important role in the reflex activation of pulmonary vagal motoneurones. Little is known about the transmitters involved in the regulation of these neurones; however, preliminary data in guinea-pigs (Bootle *et al.*, 1995) support this role of 5-HT$_{1A}$ receptors. Further, in man monoamine oxidase-A inhibition has been shown to potentiate capsaicin-induced reflex bronchoconstriction (Choudry *et al.*, 1993) which suggests the involvement of 5-hydroxytryptaminergic nerves in the reflex activation of these neurones in man, although, these data do not preclude the involvement of noradrenergic pathways.

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