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# Prostaglandin $E_2$ suppression of acetylcholine release from parasympathetic nerves innervating guinea-pig trachea by interacting with prostanoid receptors of the $EP_3$-subtype

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**1** We have demonstrated recently that exogenous prostaglandin $E_2$ ($PGE_2$) inhibits electrical field stimulation (EFS)-induced acetylcholine (ACh) release from parasympathetic nerve terminals innervating guinea-pig trachea. In the present study, we have attempted to characterize the pre-junctional prostanoid receptor(s) responsible for the inhibitory action of $PGE_2$ and to assess whether other prostanoids modulate, at a prejunctional level, cholinergic neurotransmission in guinea-pig trachea. To this end, we have investigated the effect of a range of both natural and synthetic prostanoid agonists and antagonists on EFS-evoked [$^3$H]-ACh release.

**2** In epithelium-denuded tracheal strips pretreated with indomethacin (10 $\mu$M), $PGE_2$ (0.1 nM – 1 $\mu$M) inhibited EFS-evoked [$^3$H]-ACh release in a concentration-dependent manner with an $EC_{50}$ and maximal effect of 7.62 nM and 74% inhibition, respectively. Cicaprost, an IP-receptor agonist, $PGF_{2\alpha}$ and the stable thromboxane mimetic, U46619 (each at 1 $\mu$M), also inhibited [$^3$H]-ACh release by 48%, 41% and 35%, respectively. $PGD_2$ (1 $\mu$M) had no significant effect on [$^3$H]-ACh release.

**3** The selective TP-receptor antagonist, ICI 192,605 (0.1 $\mu$M), completely reversed the inhibition of cholinergic neurotransmission induced by U-46619, but had no significant effect on similar responses effected by $PGE_2$ and $PGF_{2\alpha}$.

**4** A number of EP-receptor agonists mimicked the ability of $PGE_2$ to inhibit [$^3$H]-ACh release with a rank order of potency: GR63799X ($EP_3$-selective) > $PGE_2$ > M&B 28,767 ($EP_3$ selective) > 17-phenyl-$\omega$-trinor $PGE_2$ ($EP_1$-selective). The $EP_2$-selective agonist, AH 13205 (1 $\mu$M), did not affect EFS-induced [$^3$H]-ACh release.

**5** AH6809 (10 $\mu$M), at a concentration 10 to 100 times greater than its $pA_2$ at DP-, $EP_1$- and $EP_2$-receptors, failed to reverse the inhibitory effect of $PGE_2$ or 17-phenyl-$\omega$-trinor $PGE_2$ on [$^3$H]-ACh release.

**6** These results suggest that $PGE_2$ inhibits [$^3$H]-ACh release from parasympathetic nerves supplying guinea-pig trachea via an interaction with prejunctional prostanoid receptors of the $EP_3$-receptor subtype. Evidence for inhibitory prejunctional TP- and, possibly, IP-receptors was also obtained although these receptors may play only a minor role in suppressing [$^3$H]-ACh release when compared to receptors of the $EP_3$-subtype. However, the relative importance of the different receptors will depend not only on the sensitivity of guinea-pig trachea to prostanoids but on the nature of the endogenous ligands released locally that have activity on parasympathetic nerves.

**Keywords:** Cholinergic neurotransmission; EP-receptors; acetylcholine release; trachea

## Introduction

The parasympathetic innervation provides the primary means by which airways tone is regulated in guinea-pigs and man (Taylor *et al*., 1984; Barnes, 1993). In the airways, cholinergic neurotransmission is modulated by a number of different prejunctional receptors, the activation of which can facilitate or inhibit the release of acetylcholine (ACh) (Barnes, 1994).

The intracellular signal transduction mechanism(s) by which activation of prejunctional receptors alters neurotransmitter release from parasympathetic nerves are still uncertain. However, it is widely accepted that an increase in intracellular adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP) facilitates the exocytosis of ACh from motor nerves (Wilson, 1974; Standaert & Dretchen, 1979; Wessler & Anschutz, 1988). These observations are consistent with the recent demonstration that cyclic AMP-elevating drugs enhance the release of ACh from cholinergic nerve endings innervating the airways (Zhang *et al*., 1995, 1996; Belvisi *et al*., 1996). Indeed, previous studies from our laboratory have demonstrated that isoprenaline, an established activator of adenylyl cyclase, and a variety of cyclic AMP-elevating drugs facilitate the release of [$^3$H]-ACh from parasympathetic nerves innervating guinea-pig trachea in response to electrical field stimulation (EFS). However, under the same experimental conditions prostaglandin $E_2$ ($PGE_2$), which like isoprenaline stimulates cyclic AMP synthesis in the vagus nerve (Roch & Salamin, 1977; Kalix, 1979), inhibited rather than enhanced EFS-evoked [$^3$H]-ACh release (Belvisi *et al*., 1996). While this finding is consistent with the inhibitory effect of $PGE_2$ on EFS-evoked [$^3$H]-ACh release, and on the contractile responses elicited by EFS in the airways of many species (Nakanishi *et al*., 1978; Jones *et al*., 1980; Walters *et al*., 1984; Inoue *et al*., 1984; Black *et al*., 1989; Deckers *et al*., 1989; Ito *et al*., 1990; DeLisle *et al*., 1992; Ellis & Conanan, 1996), the observation is paradoxical since $PGE_2$ and isoprenaline evoke opposite effects on [$^3$H]-ACh release.

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Therefore, we hypothesized that $PGE_2$ either increases cyclic AMP in non-neuronal cells (e.g glia, schwann cells) that constitute the vagus nerve, or suppresses cholinergic neurotransmission via interacting with an EP-receptor subtype distinct from that which is positively coupled to adenylyl cyclase. Indeed, it is now known that distinct receptors for the naturally occurring prostanoids exist which have been given the prefix DP, EP, FP, IP and TP (see Coleman *et al*., 1994, for review). Furthermore, there is now pharmacological evidence for the existence of at least four subtypes of the EP-receptor and three of these ($EP_1$, $EP_2$, $EP_3$) have now been cloned and expressed (see Coleman *et al*., 1994, for review).

The aim of the present study was to characterize the prejunctional inhibitory prostanoid receptor(s) present on parasympathetic cholinergic nerve terminals which could be responsible for inhibiting cholinergic neurotransmission in guinea-pig trachea. Since quantitative measurement of ACh overflow from nerve endings is the only direct method to demonstrate unequivocally the occurrence of a prejunctional modulation of cholinergic neurotransmission, we have investigated the effect of a range of naturally occurring and synthetic prostanoids agonists and antagonists on EFS-evoked [$^3$H]-ACh release from parasympathetic nerves in guinea-pig trachea.

A preliminary account of some of these observations has been presented to the American Society of Pharmacology and Experimental Therapeutics (Belvisi *et al*., 1997).

## Methods

### *Preparation of guinea-pig trachea*

Male Dunkin-Hartley guinea-pigs (Harlan-Olac) (300 – 500 g) were killed by cervical dislocation and the tracheal tissue prepared as described previously (Belvisi *et al*., 1996). The lungs, with trachea and bronchi attached, were rapidly removed and placed in oxygenated Krebs-Henseleit solution (KHS) of the following composition (in mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaH_2PO_4$ 1.2, $NaHCO_3$ 25.5 and glucose 5.6. The trachea was dissected away from the lungs and main bronchi, and opened longitudinally by cutting through the cartilage; the epithelium was subsequently removed by careful dissection with minimal damage to the smooth muscle. Indomethacin (10 $\mu$M) was present throughout to prevent the formation of endogenous prostaglandins which have been demonstrated to affect cholinergic neurotransmission and [$^3$H]-ACh release *per se* (Walters *et al*., 1984; Deckers *et al*., 1989; Wessler *et al*., 1994; Belvisi *et al*., 1996).

### *Measurement of [$^3$H]-ACh release from parasympathetic nerves*

The release of [$^3$H]-ACh from cholinergic nerves was measured as described by Patel *et al*., (1995). Briefly, eight strips of smooth muscle with the cartilage and epithelium removed were studied in parallel. Each tissue was connected top and bottom with silver wire and mounted in a jacketed chamber. Tissues were superfused (Watson-Marlow model 503S; Smith and Nephew, Falmouth) at a rate of 1 ml $min^{-1}$ throughout the experiment with oxygenated KHS (pH 7.4) maintained at 37°C. The tissues were allowed to equilibrate for 30 min during which time they were continuously superfused with KHS. EFS (40 V, 0.5 ms pulse width, 4 Hz) was applied continuously for the last 10 min delivered via the silver wire electrodes. Tissues were then placed into vials containing 1.5 ml of oxygenated KHS supplemented with [$^3$H]-choline (67 nM; specific radioactivity: 2.78 TBq/$mmol^{-1}$) and EFS was applied (40 V, 0.5 ms pulse width, 4 Hz) for 45 min in order to facilitate uptake of [$^3$H]-choline into cholinergic nerve terminals. At the end of this period, tissues were superfused with KHS containing hemicholinium-3 (10 $\mu$M) to prevent the re-uptake of unlabelled choline into the nerves. Preparations were washed for 2 h before the beginning of the experiment to achieve a stable baseline of tritium release. During this period the superfusate was discarded. It has been shown previously that most of the tritium outflow evoked by EFS of epithelium-containing trachea is [$^3$H]-phosphorylcholine in addition to [$^3$H]-ACh, whereas EFS of epithelium-denuded tracheal preparations does not elicit significant release of [$^3$H]-phosphorylcholine (Wessler *et al*., 1990). Furthermore, the release of [$^3$H]-ACh following EFS of guinea-pig trachea is better maintained in the presence of indomethacin (D'Agnostino *et al*., 1990). Accordingly, in the studies described herein epithelium-free tissue preparations were used and indomethacin (10 $\mu$M) was present throughout (Ward *et al*., 1993; Patel *et al*., 1995).

EFS (40 V, 0.5 ms pulse width, 4 Hz for 1 min) was applied to each tissue and 1 ml fractions were collected every minute for 3 min before, 1 min during and 3 min after stimulation, and at 5 min intervals outside these times. Previous studies in this laboratory have confirmed that the tritium released during EFS under the aforementioned conditions is frequency-dependent and tetrodotoxin-sensitive and is, therefore, neuronal in origin (Ward *et al*., 1993). Furthermore ACh, at a concentration that evoked a contraction of a similar magnitude to that elicited by EFS with similar stimulation parameters, does not evoke the release of [$^3$H]-ACh by merely contracting the tissue (Ward *et al*., 1993). Drugs were added to the KHS superfusing each tissue after one control EFS, as detailed in the text and figure legends. A test EFS was then applied 15 and 30 min after addition of the drugs as indicated. When the effect of an antagonist was evaluated, it was added to the superfusing KHS 30 min before the third stimulation in the presence of the agonist. In the absence of exogenous agents, the release of [$^3$H]-ACh was reproducible over at least three consecutive periods of EFS ($473.2 \pm 75.9\%$, $510.9 \pm 142.3\%$ and $491.3 \pm 130.4\%$ increase in tritium overflow after the first, second and third stimulation, respectively, $n = 8$).

At the end of the experiment tissues were solubilized by treatment with 1 ml Soluene (Canberra Packard, Pangbourne, U.K.) and were counted together with aliquots of each fraction in 4 ml scintillant (Pico-Fluor 40; Canberra Packard). After determination of radioactivity, the fractional release of tritium from each preparation was calculated as a rate coefficient of each collection period at the mid point time (Patel *et al*., 1995). The increase in tritium overflow evoked by EFS was expressed as a percentage increase in the rate coefficient during the period of EFS over the average release for the preceding 3 min control period.

### *Drugs, chemicals and analytical reagents*

The following drugs were obtained from the Sigma Chemical Company (Poole, Dorset, U.K.): indomethacin, hemicholinium-3, $PGE_2$, $PGD_2$, $PGF_{2\alpha}$ and U-46619 (11$\alpha$,9$\alpha$-Epoxymethano-15*S*-hydroxy-prosta-5Z,13E-dienoicacid). GR63799X ([1*R*-[1$\alpha$(Z),2($\beta$R*),3$\alpha$]]-4-(benzoylamino) pheny-l7-[3-hydroxy-2-(2-hydroxy-3-phenoxypropoxy)-5-oxocyclopentyl]-4-heptenoate), AH13205 ((±)-*trans*-2-[4-(1-hidroxyhexyl)phenyl]-5-oxocyclopentaneheptanoic acid) and AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) were donated by Glaxo-Wellcome (Stevenage, U.K.). Cicaprost was kindly provided

by Schering Aktiengesellschaft (Berlin, Germany) and M&B 28,767 ((±)15α-hydroxy-9-oxo-16-phenoxy-17,18,19,20-tetranorprost-13-trans-enoic acid) was from Rhône-Poulenc Rorer (Dagenham, Essex). ICI 192,605 (4(Z)-6-(2-*0*-chlorophenyl-1,3-dioxan-*cis*-5-yl) hexenoic acid), 17-phenyl-ω-trinor $PGE_2$ and [methyl-$^3$H]-choline chloride (37 Ci mmol$^{-1}$) were from Zeneca (Macclesfield, Cheshire), Cayman Chemical Company (Bingham, Nottingham) and Amersham International (Amersham, Buckinghamshire), respectively.

All drugs were made up daily and dissolved in distilled water except the following: indomethacin was made up at 1 mg ml$^{-1}$ in phosphate buffer (in mM): $KH_2PO_4$ 20, $Na_2HPO_4$ 120 (pH 7.8); $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, U-46619, ICI 192,605, 17-phenyl-ω-trinor, GR63799X and M&B 28,767 were made up in a 10 mM stock solution in 100% ethanol; AH6809 and AH13205 were diluted in a solution containing 0.1% $NaHCO_3$ in 0.9% saline.

### *Statistical analysis*

Data are expressed as mean ± s.e.mean of $n$ independent observations. In all experiments each tissue acted as its own control and the results obtained before and after drug treatment were compared by Wilcoxon's rank order test for paired data. Concentration-response curves were analysed by least squares, non-linear iterative regression with the 'PRISM' curve fitting programme (GraphPad Instat software programme, San Diego, CA, U.S.A.) and $EC_{50}$ values were subsequently interpolated from curves of best fit. Equieffective molar concentration ratios (e.c.r) were calculated using the formula: $EC_{50}$ (EP-receptor analogue)/$EC_{50}$ ($PGE_2$). The null hypothesis was rejected when $P<0.05$.

## Results

### *Effect of prostanoids on EFS-induced [$^3$H]-ACh release*

$PGE_2$ (0.1 nM – 1 μM) inhibited EFS-evoked [$^3$H]-ACh release in a concentration-dependent manner with a maximal inhibition of 74 ± 4% ($P>0.01$; $n=13$) and an $EC_{50}$ of 7.62 nM (Figures 1 and 2). $PGF_{2\alpha}$, the IP-receptor agonist, cicaprost, and the stable thromboxane mimetic, U-46619, also inhibited EFS-evoked [$^3$H]-ACh release in a concentration-dependent manner, but were much less potent then $PGE_2$ producing a 41.4 ± 4.9% ($P<0.05$; $n=7$), 48.1 ± 10 % ($P<0.05$; $n=6$) and 35.7 ± 6.5% ($P<0.05$; $n=6$) inhibition respectively at 1 μM (Figure 1). $PGD_2$ (1 μM) had no significant effect on EFS-induced [$^3$H]-ACh release. Furthermore, there was no change in tritium efflux evoked by EFS in vehicle treated tissues (1.2 ± 6.9% inhibition, NS, $n=7$) (Figure 1).

### *Effect of the selective TP-receptor antagonist ICI 192,605, on the inhibition of [$^3$H]-ACh release induced by U46619, $PGE_2$ and $PGF_{2\alpha}$*

To establish whether TP-receptors are involved in mediating the inhibition of EFS-evoked [$^3$H]-ACh release elicited by $PGE_2$, $PGF_{2\alpha}$ and U46619, tissues were pretreated with the TP receptor antagonist, ICI 192,605 (Brewster *et al*., 1988; 0.1 μM) for 30 min before the third EFS. As shown in Figure 3, ICI 192,605 completely antagonized the inhibitory effect of U 46619 (1 μM) but failed to block the same response produced by $PGE_2$ (1 μM, 76.1 ± 10.9% inhibition before and 82.2 ± 2.7% inhibition after the antagonist, NS, $n=6$) and $PGF_{2\alpha}$, (1 μM, 32.8 ± 8.4% inhibition before and 38.1 ± 8.2% inhibition after the antagonist, NS, $n=6$). ICI 192,605 (0.1 μM) *per se* did not alter the EFS-evoked output of [$^3$H]-ACh (18.8 ± 15% inhibition, NS, $n=6$).

### *Effect of $PGE_2$ and selective EP-receptor agonists on EFS-induced [$^3$H]-ACh release*

The selective $EP_3$-receptor agonists GR63799X (Bunce *et al*., 1990; 0.1 nM – 1 μM) and M&B 28,767 (Lawrence *et al*., 1992; 1 nM – 1 μM), and the selective $EP_1$-receptor agonist 17-phenyl-ω-trinor $PGE_2$ (Lawrence *et al*., 1992; 1 nM – 10 μM) inhibited EFS-evoked [$^3$H]-ACh release in a concentration-dependent fashion (Figure 4). GR63799X was sevenfold more potent than $PGE_2$, whereas M&B 28,767 and 17-phenyl-ω-trinor $PGE_2$ were 2 and 13 fold less potent than $PGE_2$, respectively. The selective $EP_2$ agonist, AH13205 (Nials *et al*., 1993; 1 μM), had no significant effect on EFS-induced [$^3$H]-ACh release. The rank order of potency for the EP agonists tested was: GR63799X > $PGE_2$ > M&B 28,767 > 17-phenyl-ω-trinor $PGE_2$ > AH13205. Respective $EC_{50}$ and e.c.r values are shown in Table 1. In all cases the vehicle used (0.1% ethanol, which

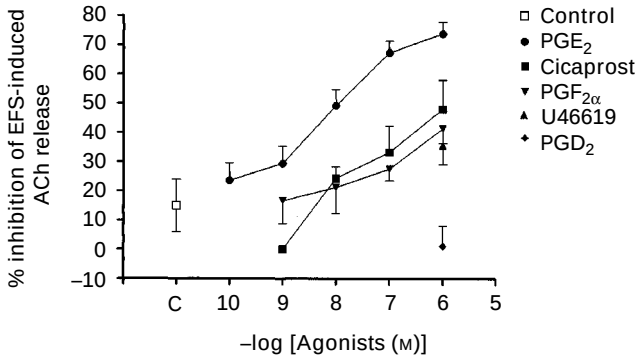


**Figure 1** Effect of prostanoids on EFS (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-induced [$^3$H]-ACh release from guinea-pig tracheal strips. Concentration-response curves were constructed to $PGE_2$ (0.1 nM – 1 μM), cicaprost (1 nM – 1 μM), $PGF_{2\alpha}$ (1 nM – 1 μM), $PGD_2$ (1 μM) and U-46619 (1 μM). C denotes the effect of the vehicle (0.1% ethanol which was the diluent for the highest concentration of the prostanoids used) on EFS-induced [$^3$H]-ACh release. Data were derived by comparing neurotransmitter release evoked by the first and second (with agonist) EFS. Values represent the mean and vertical lines s.e.mean of 6 to 9 independent determinations.

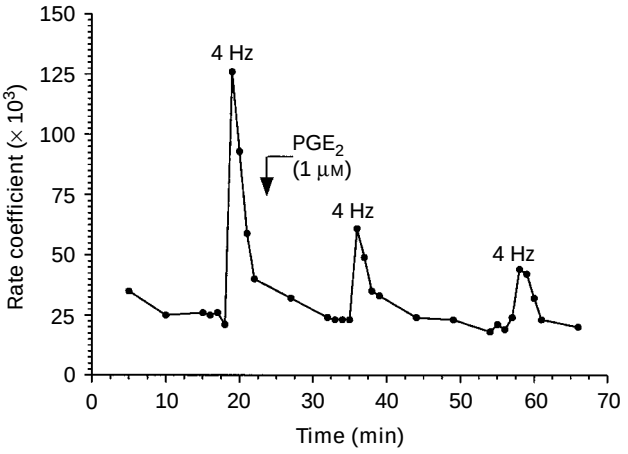


**Figure 2** Inhibition by $PGE_2$ (1 μM) of EFS (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-induced ACh release from an individual tracheal strip. The results are expressed as the rate coefficient which is a measure of the fractional $^3$H-release plotted against the time (min).

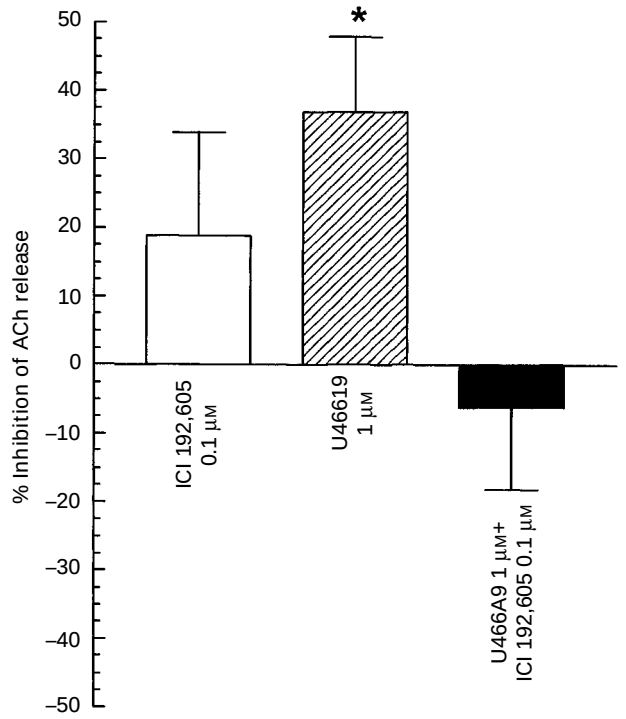


**Figure 3** Antagonism by the selective TP-receptor antagonist ICI 192, 605 (0.1 $\mu$M) of the inhibitory effect of U-46619 (1 $\mu$M) on EFS (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-induced [$^3$H]-ACh release from guinea-pig trachea. Each column shows the percentage change in the response after drug administration compared to the first control stimulation, and represents the mean $\pm$ s.e.mean of six to nine independent observations. $^*P<0.05$ compared with control values preceding drug administration.

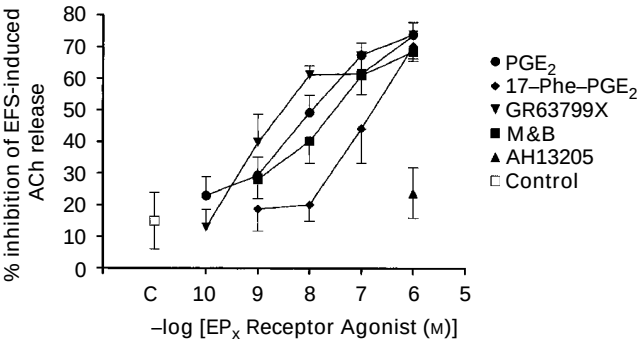


**Figure 4** Effect of $PGE_2$ and selective EP-receptor agonists on EFS (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-induced [$^3$H]-ACh release from guinea-pig tracheal strips. Concentration-response curves were constructed to $PGE_2$ (0.1 nM – 1 $\mu$M), GR 63799X (0.1 nM – 1 $\mu$M), M&B 28,767 (1 nM – 1 $\mu$M), 17-phenyl-$\omega$-trinor $PGE_2$ (1 nM – 10 $\mu$M) and AH 13205 (1 $\mu$M). C denotes the effect of vehicle. Values represent mean and vertical lines s.e.mean of 6 to 12 independent determinations. See Methods and legend to Figure 1 for further details.

was the diluent for the highest concentration) did not significantly alter EFS-evoked [$^3$H]-ACh output.

### *Effect of the DP-, $EP_1$ and $EP_2$-receptor antagonist, AH6089, on the inhibition induced by $PGE_2$ and 17-pheny-$\omega$-trinor $PGE_2$*

Tissues were pretreated with the DP-, $EP_1$ and $EP_2$-receptor, antagonist AH 6089 (Coleman *et al*., 1987; Keery & Lumley *et al*., 1988; Woodward *et al*., 1995; 10 $\mu$M), for 30 min before the third EFS. AH 6089 failed to reduce the inhibitory effect evoked by 1 $\mu$M $PGE_2$ (87.2 $\pm$ 2.4% inhibition before and 75.3 $\pm$ 4.3% inhibition after the addition of the antagonist, $n=6$) and 1 $\mu$M 17-phenyl-$\omega$-trinor $PGE_2$ (48.8 $\pm$ 10% inhibition before and 66.9 $\pm$ 12 inhibition after the antagonist, $n=6$) on EFS-evoked [$^3$H]-ACh release. AH 6809 (10 $\mu$M) *per se* did not significantly effect EFS-induced-[$^3$H]-ACh release when used alone under identical experimental conditions.

**Table 1** Relative potencies of the selective EP-receptor agonists in inhibiting EFS-evoked ACh release from parasympathetic nerves innervating guinea-pig trachea

| *Agonist* | $EC_{50}$ (nM) | *Relative potency* (e.c.r. $PGE_2=1$) |
|---|---|---|
| $PGE_2$ | 7.62 | 1 |
| GR63799X | 1.1 | 0.14 |
| M&B 28, 767 | 14.4 | 1.9 |
| 17-Phenyl-trinor $PGE_2$ | 97.9 | 12.8 |
| AG 13205 | Inactive | – |

$EC_{50}$ values were determined from the concentration-response curves shown in Figure 4.

## Discussion

The aim of this study was to identify the prejunctional prostanoid receptors which modulate ACh release from parasympathetic nerves innervating guinea-pig trachea with particular emphasis on the EP-receptor subtype. As demonstrated previously (Belvisi *et al*., 1996), $PGE_2$ potently inhibited EFS-evoked [$^3$H]-ACh release in tracheal smooth muscle under conditions where the synthesis of endogenous prostanoid was prevented by indomethacin. These data are similar to those obtained in canine trachea and bronchi (Deckers *et al*., 1989; Wang *et al*., 1994) and, given the submicromolar potency of $PGE_2$, indicate that this response is likely to be mediated by an EP-receptor. Inhibitory TP- and, possibly, IP-receptors are also expressed by cholinergic nerve terminals (see below). However, no evidence for FP- and DP-receptors was obtained, supporting previous results obtained from experiments performed with canine airways (Deckers *et al*., 1989).

Four distinct EP-receptor subtypes have been described, and studies with naturally occurring and synthetic prostanoid agonists and antagonists were performed to classify the EP-receptor subtype(s) on the cholinergic nerve terminals that innervate guinea-pig trachea. The finding that 17-phenyl-$\omega$-trinor $PGE_2$ and AH 13205 (Nials *et al*., 1993) were greater than 12 and 100 fold less potent, respectively, compared with $PGE_2$, provides persuasive evidence that this preparation does not express $EP_1$- or $EP_2$-receptors that negatively regulate [$^3$H]-ACh release. Further support for this conclusion was the failure of the $EP_1$-/$EP_2$-/DP-receptor antagonist, AH 6809 (Coleman *et al*., 1987; Keery & Lumley, 1988; Woodward *et al*., 1995) to antagonize the effect of $PGE_2$ when used at a concentration 10 to 100 times greater than the $pA_2$ at these receptors (Coleman *et al*., 1994). However, it should be noted that the $EP_2$-receptor antagonist activity of AH6809 has only been demonstrated at the human form of the $EP_2$-receptor (Woodward *et al*., 1995).

The high potency of $PGE_2$ compared to the other prostanoids tested, insensitivity to the TP-receptor antagonists, ICI 192,605, together with the activity profile of a range of EP-receptor agonists suggests that prejunctional $EP_3$-

receptors mediate the inhibitory effect of $PGE_2$ on EFS-induced [$^3$H]-ACh release. Primary evidence to support this assertion derives from the rank order of potency of selective EP-receptor agonists. Thus GR 63799X, a highly selective agonist at the $EP_3$-subtype, was seven times more potent than $PGE_2$ at suppressing [$^3$H]-ACh release which is consistent with studies performed in other $EP_3$-containing tissues such as the guinea-pig vas deferens (Bunce *et al*., 1990). Another selective $EP_3$-receptor agonist, M&B 28,767, also inhibited EFS-induced [$^3$H]-ACh release and, in agreement with the data obtained in the guinea-pig ileum (Lawrence *et al*., 1992), was approximately equi-effective with $PGE_2$. While these results provide strong persuasive evidence for prejunctional $EP_3$-receptors that inhibit [$^3$H]-ACh release in guinea-pig trachea, confirmation of this conclusion will have to await the development of selective EP-receptor antagonists.

$PGE_2$ was shown previously to modulate noradrenergic and cholinergic neurotransmission from central and peripheral nerves in many species. Moreover, many investigators have demonstrated the existence of prejunctional EP-receptors on postganglionic sympathetic nerve terminals. For example, in rat vena cava (Molderings *et al*., 1992), human saphenous vein and human pulmonary artery (Molderings *et al*., 1994), EFS-evoked [$^3$H]-noradrenaline release is inhibited by $PGE_2$ through a prejunctional $EP_3$-receptor. Similar conclusions have been reached with rat trachea (Racke *et al*., 1992) and guinea-pig atrium (Mantelli *et al*., 1991). Furthermore, a prejunctional $EP_3$-receptor modulates the $PGE_2$-induced inhibition of [$^3$H]-dopamine release from rabbit retina (Al-Zadjali *et al*., 1994). Taken together, these observations strongly suggest that prejunctional $EP_3$-receptors are ubiquitously expressed on autonomic nerve varicosities and, when activated, suppress neurotransmitter release.

The signal transduction pathway(s) that is recruited following activation of $EP_3$-receptors in nerve terminals is unclear. In many tissues, including renal collecting tubule epithelial cells (Sonnesburg & Smith, 1988), rat stomach (Reeves *et al*., 1988) and rabbit retina (Ohaia *et al*., 1995), agonism at $EP_3$-receptors results in a pertussis toxin-sensitive inhibition of adenylyl cyclase. However, although a decrease in cyclic AMP is a plausible mechanism that could account for the inhibition of neurotransmitter release, $EP_3$ receptors can also couple positively to adenylyl cyclase, via Gs, and to phospholipase C via Gq and $G_{11}$ (An *et al*., 1994; Kotani *et al*., 1995; Namba *et al*., 1993). This heterogeneity of potential coupling mechanisms is explained to some extent, by the expression of splice variants of the $EP_3$ receptor. Indeed, the cloning of an EP receptor from a bovine adrenal gland cDNA library revealed that the $EP_3$ mRNA undergoes alternative splicing to generate at least four subtypes ($EP_{3A}$, $EP_{3B}$, $EP_{3C}$, $EP_{3D}$) (Namba *et al*., 1993), although up to seven distinct transcripts are possible (Regan *et al*., 1994). These receptor isoforms show almost identical ligand-binding specificities but couple to different G-proteins and to the activation of different signalling pathways. Of importance is the fact that $EP_3$ receptor multiplicity is also present in the mouse (Irie *et al*., 1993) and in man (Adam *et al*., 1994; Regan *et al*., 1994) indicating that it probably occurs in many species.

The results obtained with cicaprost (Sturzenbechner *et al*., 1986) suggest that IP-receptors are also expressed on cholinergic nerve terminals and mediate inhibition of ACh release. Although no antagonists are currently available with which to confirm this assertion, the knowledge that cicaprost is a highly selective IP receptor agonist (Dong *et al*., 1986) is consistent with this interpretation. Nevertheless, the possibility remains that the effect of cicaprost could also result from a weak action at $EP_3$-receptors.

$PGF_{2\alpha}$ has appreciable activity at TP- and EP-receptors and we sought to determine the receptor that mediates the inhibitory effect of this prostanoid on [$^3$H]-ACh release. Since the selective TP receptor antagonist ICI 192,605 failed to inhibit the $PGF_{2\alpha}$-induced inhibition, it is unlikely that TP-receptors are involved. Therefore, by exclusion, it is possible that $PGF_{2\alpha}$ suppresses EFS-induced [$^3$H]-ACh release by interacting with the prejunctional EP- or FP-receptor. However, the latter possibility is unlikely since $PGF_{2\alpha}$ is arguably the least selective of the naturally occurring prostanoids and, in the guinea-pig trachea, probably acts at the same receptor as $PGE_2$.

The stable thromboxane $A_2$ mimetic, U46619, weakly suppressed EFS-evoked [$^3$H]-ACh release but its action was inhibited by the selective TP-receptor antagonist, ICI 192,605, suggesting that TP-receptors can also negatively regulate cholinergic neurotransmission in this preparation. Curiously, these data contrast with results obtained in canine airways where U-46619 has been shown to enhance cholinergic neurotransmission (Chung *et al*., 1985; Serio & Daniel, 1988; Janssen & Daniel, 1991). However, in those studies ACh release was measured indirectly by comparing the effect of U-46619 on EFS-evoked twitch responses with contractions produced by exogenous ACh. As that method does not distinguish unambiguously between a pre- or a postjunctional mechanism of action, a comparison with this study, which provides a direct measure of ACh output, is inappropriate.

In conclusion, the results of this study provide evidence that $PGE_2$ inhibits cholinergic neurotransmission in the guinea-pig trachea by interacting with prejunctional prostanoid receptors of the $EP_3$-subtype. Evidence for the presence of prejunctional TP- and possibly IP-receptors on postganglionic parasympathetic nerve terminals was also provided. Under physiological or pathophysiological conditions, the role of these receptors in regulating neurotransmitter release will depend ultimately on the endogenous prostanoid released in the proximity of the parasympathetic nerve endings. The identification of $EP_3$ receptors on cholinergic nerve terminals that innervate guinea-pig trachea suggests that $PGE_2$ inhibits neurotransmission by a mechanism that probably does not involve the stimulation of adenylyl cyclase. This explanation adequately accounts for the opposite effects that $PGE_2$ exerts on EFS-induced [$^3$H]-ACh release compared to other cyclic AMP-elevating agents (Belvisi *et al*., 1996).

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