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European Journal of Pharmacology 488 (2004) 127–135
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In vivo pertussis toxin treatment reduces contraction of rat resistance arteries but not that of mouse trachea

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Received 18 August 2003; received in revised form 26 January 2004; accepted 3 February 2004

Abstract

In order to develop an in vitro method for detecting residual pertussis toxin activity in acellular pertussis vaccines, the effects of in vivo pertussis toxin treatment on contraction and relaxation properties of isolated mouse trachea and of isolated rat small mesenteric resistance arteries were studied. In vivo pertussis toxin treatment (24 or 72 μg/kg, intraperitoneally (i.p.)) did not affect contraction and relaxation properties of isolated BALB/c or NIH mouse trachea. In vivo pertussis toxin treatment (30 μg/kg, intravenously) significantly reduced noradrenaline- or KCl-induced maximal contraction and reduced sensitivity to noradrenaline in isolated male Wistar rat small mesenteric resistance arteries. However, in vivo pertussis toxin treatment did not affect relaxation properties of isolated rat small mesenteric resistance arteries. These results support the hypothesis that vasoconstriction-regulating mechanisms and not airway constriction mechanisms are involved in pertussis toxin-induced histamine sensitisation. The vasoconstriction-regulating mechanisms may provide a lead for further development of an in vitro method for measuring biologically active pertussis toxin in acellular pertussis vaccines based on mechanisms involved in the histamine sensitisation test.

Keywords: Histamine sensitisation; Pertussis toxin; Trachea; Small mesenteric resistance arteries; (Rat); (Mouse)

1. Introduction

Pertussis toxin is one of the major toxic components of *Bordetella pertussis* whole-cell vaccines. It has been demonstrated that pertussis toxin is crucial for the development of immunity to whooping cough (Cherry, 1996; Pittman, 1979). Hence, a certain level of toxicity of pertussis toxin is accepted in order to achieve a sufficient level of immunity (Gupta et al., 1988). Pertussis toxin is characterised by its wide range of physiological activities in various animal species, including histamine sensitisation, leukocytosis promotion and enhancement of insulin secretion by activation of the islets of Langerhans (Nogimori et al., 1985; Parfentjev and Goodline, 1948). Furthermore, pertussis toxin produces autonomic and haemodynamic impairment (De Wildt et al., 1982, 1983, 1985, 1986; Van Amsterdam et al., 1998a,b; Vleeming et al., 1993).

The acellular vaccines are a new generation of pertussis vaccines. Detoxified pertussis toxin is used in contrast to whole-cell pertussis vaccines (Anderson et al., 1994; Edwards and Karzon, 1990; Robinson and Funnell, 1992; Wardlaw, 1992). Its adverse effects make detoxification of pertussis toxin essential. A safety test in mice is required by the regulatory authorities in order to confirm the absence of residual pertussis toxin toxicity or reversal of detoxified pertussis to pertussis toxin. This test is called the histamine sensitisation test as mice injected with biologically active pertussis toxin are sensitised to histamine (Parfentjev and Goodline, 1948), ultimately resulting in a decrease of the lethal dose of histamine. The test causes severe suffering to the animals involved. For this reason, we started to develop an in vitro method for detecting residual pertussis toxin toxicity, based on mechanisms involved in pertussis toxin-induced histamine sensitisation.

Since the pathophysiology of histamine sensitisation by pertussis toxin is unknown, mechanisms were studied initially to understand which substrates (e.g. receptors

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doi:10.1016/j.ejphar.2004.02.003

and/or signal transduction systems) and physiological systems (cardiovascular and pulmonary) are involved in pertussis toxin-induced histamine sensitisation. Previous studies had shown that, in addition to mice, rats could be sensitised to histamine by administration of biologically active pertussis toxin (Vleeming et al., 2000a). Administration of pertussis toxin in rats resulted in enhancement of the histamine-induced decrease in mean arterial blood pressure and decreased the lethal dose of histamine (Vleeming et al., 2000a). Furthermore, the histamine H_1-receptor, but not the histamine H_2- or H_3-receptor, seems to be involved in the pertussis toxin-induced histamine sensitisation (Vleeming et al., 2000a). In addition to the pertussis toxin-induced enhancement of the histamine-induced decrease in blood pressure, pertussis toxin also decreases diastolic blood pressure in rats (De Wildt et al., 1986; Vleeming et al., 2000a,b). This indicates a possible involvement of the vascular system in pertussis toxin-induced histamine sensitisation.

Besides a possible involvement of the vascular system in pertussis toxin-induced histamine sensitisation, the involvement of the pulmonary system in pertussis toxin-induced histamine sensitisation remains unclear (Vleeming et al., 2000a). Histamine-induced mortality in the histamine sensitisation test could be the result of histamine-induced hyperreactivity of airway smooth muscle. Moreover, pertussis toxin catalyses the transfer of an adenosine diphosphate-ribose moiety to α-subunits of signal-transducing guanine-nucleotide-binding proteins (G-proteins), leading to uncoupling of the G-protein from the corresponding receptor and loss of effector regulation (Gierschik, 1992). In this way, receptors of pulmonary and vascular smooth muscle other than the histaminergic receptors could also be involved in pertussis toxin-induced histamine sensitisation.

We hypothesise that the vascular or the pulmonary system or both are involved in pertussis toxin-induced histamine sensitisation. The objective of this study was to determine if in vivo pertussis toxin treatment interferes with the receptor-stimulated induced contraction and/or relaxation properties of isolated airway and/or vascular smooth muscle.

2. Materials and methods

2.1. Animals

Male SPF BALB/c mice, 6–8 weeks old and weighing 20–25 g, were used. They were obtained from the breed-

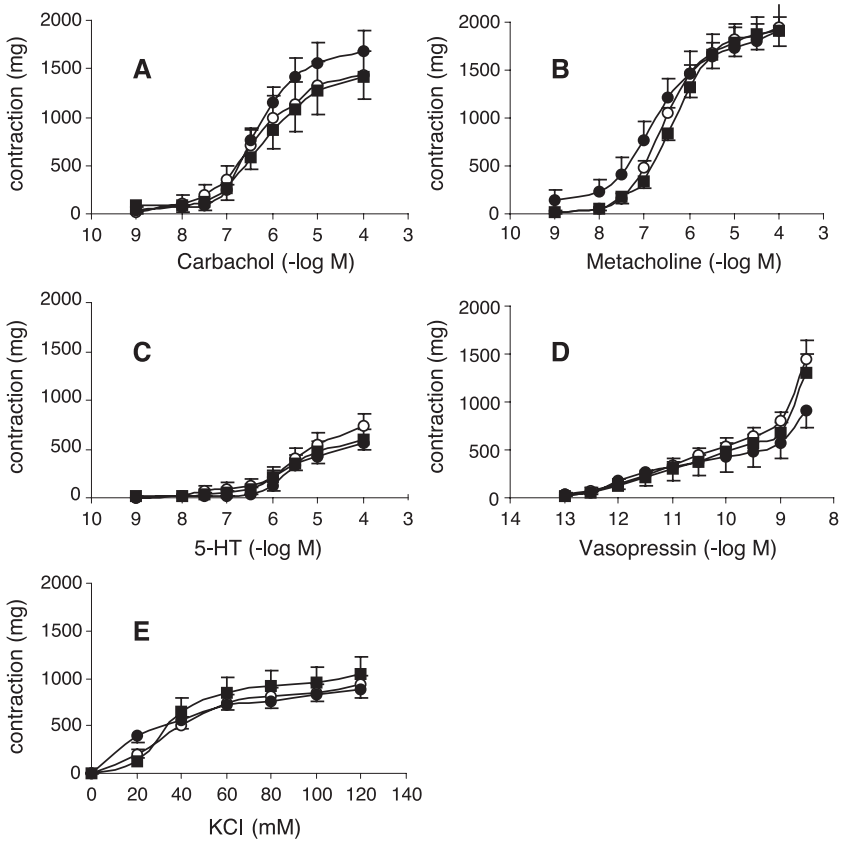

Fig. 1. Concentration–response curves for (A) carbachol, (B) methacholine, (C) serotonin, (D) vasopressin and (E) KCl in isolated tracheas from BALB/c mice treated i.p. with saline (open circles), 24 μg/kg PT (closed circles) or 72 μg/kg PT (closed squares). Each point represents the mean for 7–8 preparations ± S.E.M.

ing colony of Charles River, France. In a separate study, female SPF NIH mice, 6–8 weeks old and weighing 20–25 g, were used for comparison to take mouse strains into account. NIH mice were derived from the National Institute for Public Health and Environment (RIVM) (Bilthoven, The Netherlands).

Male SPF Wistar rats, weighing 250–350 g each, were used. They were obtained from the SPF breeding colony of Harlan, The Netherlands. The animals were SPF-housed under constant conditions, a relative humidity of 30–60% and a temperature of 22–24 °C. Food and water were available ad libitum. The experiments were reviewed and approved by the RIVM animal ethics committee.

2.2. Pertussis toxin treatment of mice and isolation of trachea

BALB/c and NIH mice were injected i.p. with 24 μg/kg, 72 μg/kg pertussis toxin (PTPU 1024) or saline. The pertussis toxin dose of 24 μg/kg was based on a previous histamine sensitisation test in NIH mice and caused a histamine-induced lethality of 50%. A three-fold higher concentration of pertussis toxin was also used, because the histamine sensitisation effect that occurs in vivo as a consequence of pertussis toxin vaccination could presents itself differently in vitro. At 5 days after pretreatment with 24 μg/kg pertussis toxin, 72 μg/kg pertussis toxin or saline, the mice were killed with an i.p. injection of 0.25 ml Nembutal (pentobarbitone sodium, 60 mg/ml). Thereafter, the thorax was opened and the trachea was isolated. The tracheas were transferred to a petri dish containing modified oxygenated Krebs' bicarbonate solution of the following composition (mM) NaCl 118.1, KCl 4.7, $CaCl_2 \cdot 6H_2O$ 2.5, $MgCl_2 \cdot 6H_2O$ 0.5, $NaHCO_3$ 25.0, $NaH_2PO_4 \cdot H_2O$ 1.0, glucose 11.1 in distilled water.

The trachea was prepared free of remaining connective tissue and fat under a binocular preparation microscope and pieces approximately 6 mm length (nine trachea rings just beneath the larynx) were taken. The tracheas were slipped onto two supports in an organ bath. One support in the organ bath was connected to an isometric transducer and recorder. The other support was connected to a fixed pin on the organ bath side. The organ bath was filled with 12.5 ml Krebs bicarbonate solution and gassed with a mixture of 95% oxygen and 5% carbon dioxide. The temperature was maintained at 37 °C with a constant temperature-circulating unit (Julabo type VC/3). Contraction and relaxation were recorded isometrically via a force-displacement transducer (Department of Pharmacology, University of Utrecht, Utrecht) and a two-channel recorder (Kipp and Sons model BD 112, Delft, the Netherlands) and are expressed as changes in grams force. Before the addition of drugs, the tracheas were equilibrated for at least 60 min under a resting tension of 1 g with replacement of the bath fluid every 15 min. The drugs were dissolved in saline and kept in the refrigerator till needed. Cumulative contractile concentration–response curves were constructed for the cholinomimetics, carbachol and methacholine, the agonists, histamine and serotonin, the bioactive peptide, vasopressin and for KCl. Cumulative relaxant concentration–response curves were made for the β-adrenoceptor agonist, isoprenaline, the agonist, histamine and sodium nitroprusside. Relaxation with isoprenaline, histamine and sodium nitroprusside was measured in tracheas precontracted with carbachol 3.0×10^{-7} M (approximately equal to the EC_{50}, Garssen et al., 1990). Stability of the precontraction was determined by adding saline instead of drugs.

2.3. Pertussis toxin treatment of rats and preparation of small mesenteric resistance arteries

Rats were injected intravenously with saline or pertussis toxin 30 μg/kg (batch PTPU 1049). At 5 days after pretreatment with pertussis toxin or saline, the rats were killed, 10 min after i.p. injection of heparin, by decapitation and exsanguination. The mesentery was removed and immersed in cold, gassed (95% O_2 and 5% CO_2) Krebs–Ringer solution (pH 7.4) of the following composition (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 24.9 and glucose 11.0. Second to third order side branches of mesenteric arteries were dissected from the mesentery and prepared free of fat and connective tissue. To exclude influences of sympathetic nerve endings on mechanical and pharmacological properties, the sympathetic

Table 1
EC_{50} values (M) and E_{max} values (mg) of contraction-inducing agonists for tracheas from control or PT-treated BALB/c mice

Agonist	Treatment	n	EC_{50}	E_{max}
Carbachol	control	8	$7.56 \times 10^{-7} \pm 3.0 \times 10^{-7}$	1429 ± 261
	24 μg/kg	8	$4.82 \times 10^{-7} \pm 0.6 \times 10^{-7}$	1687 ± 207
	PT		(n.s.)	(n.s.)
	72 μg/kg	8	$1.69 \times 10^{-6} \pm 1.1 \times 10^{-6}$	1412 ± 225
	PT		(n.s.)	(n.s.)
Methacholine	control	8	$2.99 \times 10^{-7} \pm 0.4 \times 10^{-7}$	1939 ± 124
	24 μg/kg	7	$1.79 \times 10^{-7} \pm 0.3 \times 10^{-7}$	1905 ± 272
	PT		(n.s.)	(n.s.)
	72 μg/kg	7	$4.49 \times 10^{-7} \pm 0.7 \times 10^{-7}$	1917 ± 161
	PT		(n.s.)	(n.s.)
Serotonin	control	8	$5.17 \times 10^{-6} \pm 1.7 \times 10^{-6}$	733 ± 120
	24 μg/kg	8	$4.7 \times 10^{-6} \pm 2.6 \times 10^{-6}$	556 ± 72
	PT		(n.s.)	(n.s.)
	72 μg/kg	8	$3.41 \times 10^{-6} \pm 1.0 \times 10^{-6}$	593 ± 109
	PT		(n.s.)	(n.s.)
Vasopressin[a]	control	8	–	1454 ± 196
	24 μg/kg	8	–	907 ± 183
	PT			(n.s.)
	72 μg/kg	8	–	1306 ± 190
	PT			(n.s.)
KCl	control	8	$3.7 \times 10^{-2} \pm 3.6 \times 10^{-6}$	938 ± 98
	24 μg/kg	7	$2.7 \times 10^{-2} \pm 4.2 \times 10^{-3}$	876 ± 82
	PT		(n.s.)	(n.s.)
	72 μg/kg	8	$3.9 \times 10^{-2} \pm 2.5 \times 10^{-3}$	1053 ± 179
	PT		(n.s.)	(n.s.)

Data are presented as means ± S.E.M. n indicates the number of tracheal preparations used. n.s.: not significantly different from control.

[a] Vasopressin-induced contractions did not reach a maximum; therefore, EC_{50} values were not calculated.

nerve endings in the vessels were destroyed by incubating the vessel segments for 10 min at 37 °C in a bicarbonate-free Krebs–Ringer solution (pH 4.0) that contained 300 μg/ml 6-hydroxydopamine (Aprigliano and Hermsmeyer, 1976; Boonen and De Mey, 1990). The vessel segments, about 2 mm in length, were threaded on two stainless steel wires with 40 μm diameter each and mounted horizontally as ring segments in a Mulvany-Halpern myograph (Model 500A, J.P. Trading, Aarhus, Denmark). Both ends of the first wire were attached to a displacement device; the ends of the second wire were attached to an isometric force transducer. The Krebs–Ringer solution in the myograph was maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. Segment length was determined using a microscope and graticule.

Prior to experimentation, the small mesenteric resistance arteries were stretched to their individual optimal lumen diameter for force development by "normalisation" of the internal diameter of each vessel, as described by Mulvany and Halpern (1977), meaning the arteries were set to a tension equivalent to that generated at 90% of the internal lumen diameter of the vessel at a transmural pressure of 100 mm Hg. This procedure also provides a standardised measure of the internal lumen diameter (Mulvany and Halpern, 1977).

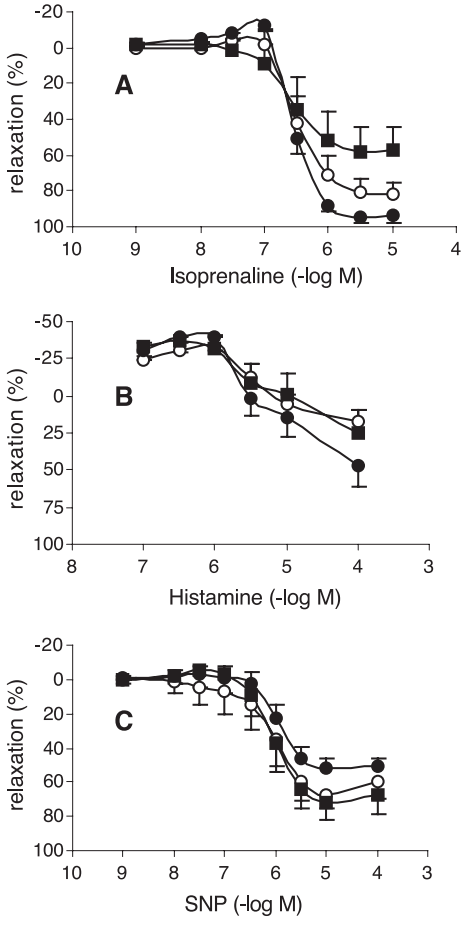

Fig. 2. Concentration–response curves for (A) isoprenaline, (B) histamine and (C) sodium nitroprusside in isolated tracheas from BALB/c mice treated i.p. with saline (open circles), 24 μg/kg PT (closed circles) or 72 μg/kg PT (closed squares). Tracheas were precontracted with 3.0×10^{-7} M carbachol. Relaxations are shown as % relaxation of precontraction. Each point represents the mean for 4–8 preparations±S.E.M.

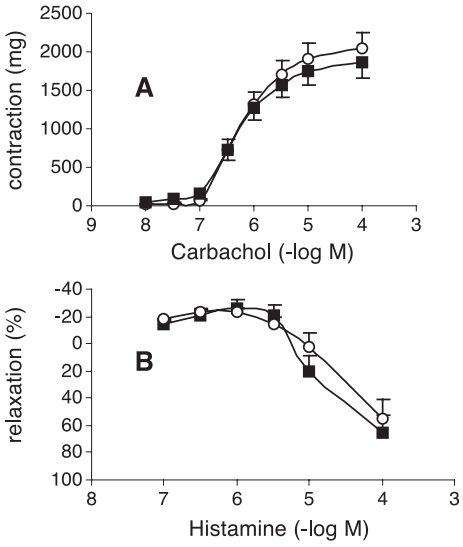

Fig. 3. Concentration–response curves for (A) carbachol and (B) histamine in isolated tracheas from NIH mice treated i.p. with saline (open circles) or 72 μg/kg PT (closed squares). Tracheas were precontracted with 3.0×10^{-7} M carbachol. Relaxation is shown as % relaxation of precontraction. Each point represents the mean for 6–8 preparations±S.E.M.

2.4. Experimental protocol for small mesenteric resistance arteries

Following an equilibration period of 30 min, small mesenteric resistance arteries were challenged three times with 120 mM KCl with interim washout periods of twice 5 min. After re-equilibration, in one set of preparations from

Table 2
EC_{50} values (M) and E_{max} values (mg) of relaxation-inducing agonists for tracheas from control or PT-treated BALB/c mice

Agonist	Treatment	n	EC_{50}	E_{max}
Isoprenaline	control	8	$4.72\times10^{-7}\pm1.4\times10^{-7}$	875 ± 100
	24 μg/kg PT	7	$3.7\times10^{-7}\pm0.6\times10^{-7}$ (n.s.)	1113 ± 120 (n.s.)
	72 μg/kg PT	6	$7.15\times10^{-7}\pm3.1\times10^{-7}$ (n.s.)	678 ± 217 (n.s.)
Histamine	control	8	$7.78\times10^{-6}\pm4.0\times10^{-6}$	557 ± 148
	24 μg/kg PT	4	$7.1\times10^{-6}\pm4.5\times10^{-6}$ (n.s.)	518 ± 47 (n.s.)
	72 μg/kg PT	7	$9.22\times10^{-6}\pm4.2\times10^{-6}$ (n.s.)	682 ± 134 (n.s.)
SNP	control	6	$1.22\times10^{-6}\pm0.4\times10^{-6}$	886 ± 189
	24 μg/kg PT	7	$1.44\times10^{-6}\pm0.3\times10^{-6}$ (n.s.)	813 ± 168 (n.s.)
	72 μg/kg PT	6	$1.36\times10^{-6}\pm0.4\times10^{-6}$ (n.s.)	827 ± 107 (n.s.)

Data are presented as means ± S.E.M. n indicates the number of tracheal preparations used. n.s.: not significantly different from control.

Table 3
EC_{50} values (M) and E_{max} values (mg) of contraction- and relaxation-inducing agonists for tracheas from control or PT-treated NIH mice

Agonist	Treatment	n	EC_{50}	E_{max}
Carbachol	control	7	$6.16 \times 10^{-7} \pm 1.1 \times 10^{-7}$	2037 ± 211
	72 μg/kg PT	8	$5.32 \times 10^{-7} \pm 0.8 \times 10^{-7}$ (n.s.)	1861 ± 192 (n.s.)
Histamine (as relaxant)	control	7	$3.05 \times 10^{-5} \pm 0.7 \times 10^{-5}$[a]	1330 ± 208[a]
	72 μg/kg PT	7	$2.91 \times 10^{-5} \pm 0.6 \times 10^{-5}$ (n.s.)	1067 ± 160 (n.s.)

Data are presented as means ± S.E.M. n indicates the number of tracheal preparations used. n.s.: not significantly different from control.
[a] $P < 0.05$, significantly different from BALB/c control group (Table 2).

control- or pertussis toxin-pretreated animals, cumulative concentration–response curves were made for noradrenaline and, after exhaustive washout and re-equilibration, for KCl. The remaining preparations were precontracted with 60 mM KCl (approximately equal to EC_{80}), after which concentration–response curves for histamine were made. Following washout of histamine and re-equilibration, small mesenteric resistance artery preparations were precontracted again with 60 mM KCl, after which a concentration–response curve for acetylcholine was performed. Stability of precontraction was evaluated by adding saline.

2.5. Pertussis toxin and drugs used

Pertussis toxin (RIVM standards PTPU 1024 and PTPU 1049) was isolated and purified from *B. pertussis* Tohama (Bilthoven, The Netherlands) and dissolved in saline before injection. Acetylcholine, [^{8}Arg]vasopressin acetate salt, carbachol (carbamylcholine chloride), histamine dihydrochloride, 6-hydroxydopamine, isoprenaline (isoproterenol) hemisulfate, methacholine chloride (acetyl-βmethylcholine chloride) and serotonin (5-hydroxytryptamine (5-HT)) creatinine sulfate complex were obtained from Sigma-Aldrich, The Netherlands. Noradrenaline was obtained from Centrafarm, the Netherlands. KCl came from Merck, Germany. Sodium nitroprusside was obtained from Bufa, The Netherlands. Nembutal was obtained from AUV, Cuijk, The Netherlands.

2.6. Data and statistical analysis

Data are presented as means ± S.E.M. All EC_{50} values, the effective concentration causing 50% of the maximal response (E_{max}), and E_{max} values were obtained from the calculation of EC_{50} and E_{max} values for each individual representative curve and then averaged ± S.E.M. Results were tested with one-way analysis of variance (ANOVA) (95%). Differences were considered to be significant when $P < 0.05$.

3. Results

3.1. Effects of in vivo pertussis toxin treatment on contraction or relaxation concentration–response curves of isolated mouse trachea

Histamine induced no contractions in tracheas from control or pertussis toxin-treated BALB/c or NIH mice (data not shown). Carbachol, methacholine, serotonin, vasopressin or KCl induced concentration-dependent contractions of isolated tracheas from control and pertussis toxin-treated BALB/c mice without significant differences in concentration–response curves (Figs. 1 and 3A). No significant

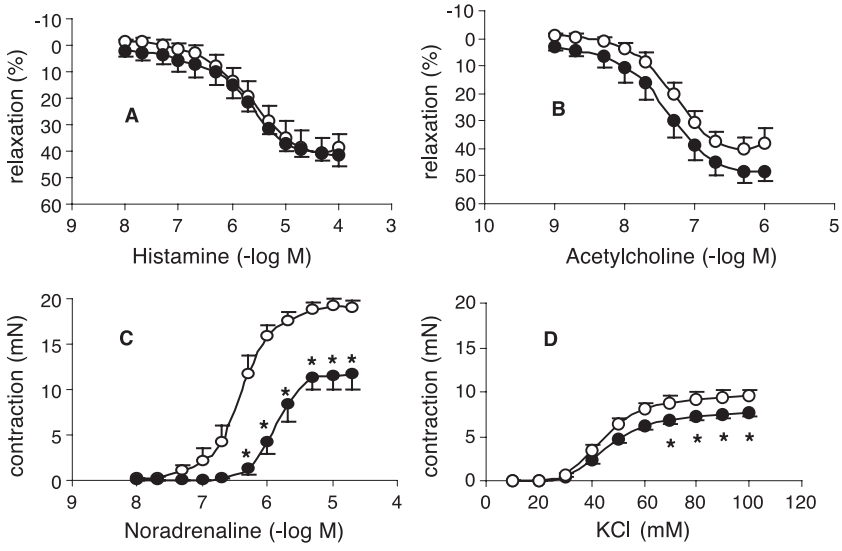

Fig. 4. Concentration–response curves for (A) histamine, (B) acetylcholine, (C) noradrenaline and (D) KCl in isolated small mesenteric resistance arteries from male Wistar rats treated i.v. with saline (open circles) or 30 μg/kg PT (closed circles). Relaxations are shown as % relaxation of precontraction. Each point represents the mean for 8–10 preparations ± S.E.M. *$P < 0.05$, significantly different from control.

differences in maximal contraction (E_{max}) and sensitivity (EC_{50}) were observed for any of the agonists between tracheas from control or pertussis toxin-treated BALB/c or NIH mice (Tables 1 and 3). Concentration–response curves for vasopressin did not reach a maximum. Therefore, EC_{50} values were not calculated (Table 1).

Tracheal rings were precontracted with 3.0×10^{-7} M carbachol. Isoprenaline, histamine or sodium nitroprusside produced concentration-dependent relaxations of the tracheal preparations isolated from control or pertussis toxin-treated BALB/c or NIH mice (Figs. 2 and 3B). No significant difference in E_{max} or EC_{50} values between groups was observed (Tables 2 and 3).

Tracheas from control NIH mice were significantly more sensitive to histamine than were tracheas from control BALB/c mice. Furthermore, the maximal relaxation response to histamine was significantly increased in tracheas from control NIH mice as compared to tracheas from control BALB/c mice (Tables 2 and 3).

3.2. Effects of in vivo pertussis toxin treatment on contraction and relaxation concentration–response curves induced by different agonists in small mesenteric resistance arteries from Wistar rats

Small mesenteric resistance arteries were precontracted with 60 mM KCl. After precontraction with KCl, histamine or acetylcholine produced concentration-dependent relaxations of the small mesenteric resistance arteries isolated from control and pertussis toxin-treated rats (Fig. 4A and B, respectively). There was no significant difference in maximal relaxation response (E_{max}) or sensitivity (EC_{50}) between small mesenteric resistance arteries from control rats and small mesenteric resistance arteries from pertussis toxin-treated rats (Table 4).

Table 4
EC_{50} values (M) and E_{max} values (mN) of contraction- and relaxation-inducing agonists for small mesenteric resistance arteries from control or PT-treated male Wistar rats

Agonist	Treatment	n	EC_{50}	E_{max}
Histamine	control	9	$3.34 \times 10^{-6} \pm 0.8 \times 10^{-6}$	3.53 ± 0.7
	30 μg/kg PT	9	$1.78 \times 10^{-6} \pm 0.5 \times 10^{-6}$ (n.s.)	2.29 ± 0.4 (n.s.)
Acetylcholine	control	9	$6.36 \times 10^{-8} \pm 1.3 \times 10^{-8}$	3.75 ± 0.7
	30 μg/kg PT	9	$4.25 \times 10^{-8} \pm 0.8 \times 10^{-8}$ (n.s.)	2.53 ± 0.3 (n.s.)
Noradrenaline	control	9	$4.82 \times 10^{-7} \pm 0.8 \times 10^{-7}$	19.25 ± 1.1
	30 μg/kg PT	8	$1.67 \times 10^{-6} \pm 0.3 \times 10^{-6}$[a]	12.25 ± 1.5[a]
KCl	control	10	$45.6 \times 10^{-3} \pm 1.6 \times 10^{-3}$	9.67 ± 0.8
	30 μg/kg PT	10	$46.2 \times 10^{-3} \pm 1.4 \times 10^{-3}$ (n.s.)	7.78 ± 0.5[a]

Data are presented as means ± S.E.M. n indicates the number of preparations used.
[a] $P < 0.05$, significantly different from control. n.s.: not significantly different from control.

The adrenoceptor agonist, noradrenaline, induced concentration-dependent contractions in isolated small mesenteric resistance arteries from control and pertussis toxin-treated Wistar rats (Fig. 4C). Pretreatment with pertussis toxin significantly reduced E_{max} and increased EC_{50} values of noradrenaline in small mesenteric resistance arteries (Table 4). Furthermore, KCl induced concentration-dependent contractions in small mesenteric resistance arteries from control and pertussis toxin-treated rats (Fig. 4D). Pretreatment with pertussis toxin also significantly reduced KCl-induced maximal contraction of small mesenteric resistance arteries (Table 4). Pretreatment with pertussis toxin did not affect sensitivity of small mesenteric resistance arteries to KCl (Table 4).

4. Discussion

Development of a mechanism based in vitro alternative for the histamine sensitisation test in mice requires an understanding of the physiological and molecular systems underlying pertussis toxin-induced histamine sensitisation. We hypothesise that the vascular or the pulmonary system or both are involved in pertussis toxin-induced histamine sensitisation. The objective of this study was to determine if in vivo pertussis toxin treatment interferes with the receptor stimulation-induced contraction and/or relaxation properties of isolated airway and/or vascular smooth muscle.

Five bronchoconstrictors were selected on the basis of different receptor selectivity. In addition, KCl was selected in order to discriminate between receptor-stimulated and aspecific contraction aspects. We showed, as have others (Garssen et al., 1990; Li et al., 1998; Richter and Sirois, 2000), that carbachol, methacholine, serotonin, vasopressin and KCl induced concentration-dependent contractions of isolated mouse trachea. Although histamine is a known constrictor of airway smooth muscle (Hill et al., 1997), histamine induced no contraction of isolated mouse trachea. This result is supported by the finding of Garssen et al. (1990) of only a very small contractile response of isolated mouse trachea to a high concentration of histamine. This suggests that histaminergic receptors do not play an important role in mouse tracheal smooth muscle contraction. Also in trachea from pertussis toxin-treated mice, histamine induced no contraction. We therefore hypothesise that the increased histamine-induced mortality of mice vaccinated with biologically active pertussis toxin cannot be caused by a histamine-induced hyperreactive contraction of pulmonary smooth muscle. The histaminergic receptors in pulmonary vascular smooth muscle are therefore considered to be unsuitable as a lead for further development of an in vitro method for detecting pertussis toxin toxicity. The present study also showed that in vivo treatment with pertussis toxin of either mouse strain affected neither the receptor-stimulated contractions nor the KCl-induced contraction of isolated tracheas. By using

two different mouse strains, which could both be sensitised to histamine by pertussis toxin (Lee et al., 1999; Van Straaten-van de Kapelle et al., 1997), we reduced the risk of strain-dependent results.

The effects of the tested agonists on isolated tracheas are known, but few studies have been published on the effect of in vivo pertussis toxin treatment on the contractility of isolated mouse tracheas. Veenendaal et al. (1986) described a hyperreactive effect of carbachol on the cholinergic system in isolated tracheas from *B. pertussis* whole-cell vaccinated mice, which appeared to be lipopolysaccharide-dependent. In addition, there are data about the effect of pertussis toxin (pre)treatment on cholinergic receptor-induced contractile responses of isolated tracheas from animal species other than mice. These results indicate an inhibitory effect of in vitro pertussis toxin treatment on contractile cholinergic receptors in porcine or guinea pig tracheal smooth muscle (Croxton et al., 1998; Hirschman et al., 1999; Kume et al., 1995). As described, we did not find an inhibitory effect of in vivo pertussis toxin treatment of mice on cholinergic receptor-induced contraction of isolated airway smooth muscle. Furthermore, other authors did not find an effect of in vivo pertussis toxin treatment of guinea pigs on contractile responses of isolated trachea to carbachol (Eglen et al., 1988). Hence, it can be suggested that, in airway smooth muscle, acetylcholine receptor transduction is affected by in vitro, but not by in vivo, treatment with pertussis toxin.

In vivo pertussis toxin treatment also did not affect the serotonin- or KCl-induced contraction of isolated tracheas from mice. In spite of the fact that vasopressin-induced contractions did not reach a maximum, it seems evident that in vivo pertussis toxin treatment does not affect vasopressin-induced concentration-dependent contractions of isolated mice tracheas. To our knowledge, no data have been published earlier that could be used for comparison.

We also investigated the effect of in vivo pertussis toxin treatment on the relaxation properties of mouse isolated tracheal smooth muscle. Histamine, isoprenaline or sodium nitroprusside caused concentration-dependent relaxations of carbachol-precontracted isolated tracheas from control or pertussis toxin-treated mice without significant differences in concentration–response curves. Although tracheas from NIH mice are more sensitive to histamine than are tracheas from BALB/c mice, no effect of in vivo pertussis toxin treatment on histamine-induced relaxation of isolated NIH mouse trachea is observed. Veenendaal et al. (1986) and Bartell and Busse (1980) described an impaired effect of *B. pertussis* whole-cell vaccination on β-adrenoceptor agonist-stimulated relaxation of isolated tracheal smooth muscle from mice, an impairment that also appeared to be lipopolysaccharide-dependent. Norris and Eyre (1982) described an impaired relaxation in response to histamine and isoprenaline of tracheal smooth muscle precontracted with carbachol after vaccination with whole-cell *B. pertussis* vaccine. Probably, these impaired agonist-induced relaxations are also caused by lipopolysaccharide and not by pertussis toxin. Furthermore, in acetylcholine-precontracted canine isolated trachea, in vitro incubation with pertussis toxin resulted in augmentation of isoprenaline-induced relaxation (Mitchell et al., 1993), in contrast with the results we found for in vivo pertussis toxin treatment. Again, this discrepancy between in vivo and in vitro pertussis toxin treatment effect on relaxation properties of isolated mouse trachea is in conflict with the aim to identify an in vivo effect of pertussis toxin and to translate it to an in vitro model. Thus, it appears that, for the tracheal receptor systems tested, in contrast to in vitro pertussis toxin treatment, in vivo pertussis toxin treatment does not uncouple pertussis toxin sensitive G-proteins from receptors.

In contrast to our observations for isolated tracheas, we found two marked inhibitory effects of in vivo pertussis toxin treatment on contractile properties of isolated rat small mesenteric resistance arteries. First, in vivo pertussis toxin treatment significantly reduced the maximum contraction induced by the adrenoceptor agonist, noradrenaline or by KCl. Secondly, in vivo pertussis toxin treatment significantly decreased the sensitivity to noradrenaline of small mesenteric resistance arteries. These results are supported by the observation that in vitro pertussis toxin treatment of rat superior mesenteric artery reduced the noradrenaline-induced maximum contraction and sensitivity to noradrenaline (Abebe et al., 1995). Furthermore, in vitro pertussis toxin treatment reduced phenylephrine-induced contraction of small mesenteric resistance arteries (Boonen and De Mey, 1990) and in vitro pertussis toxin treatment reduced the alpha-adrenoceptor-stimulated contractions of isolated rat tail artery segments (Petitcolin et al., 2001; Spitzbarth-Régrigny et al., 2000). Taken together, these findings for the inhibitory effect of pertussis toxin on the contractile properties of isolated rat small mesenteric resistance arteries provide a first lead to translate an in vivo effect of pertussis toxin to an in vitro model.

In vivo pertussis toxin treatment does not affect histamine- or acetylcholine-induced relaxation of small mesenteric resistance arteries. These observations are supported by other results (Adeagbo and Malik, 1990). It was hypothesised that (a) vasodilatation-inducing signal transduction pathway(s) were (was) involved in pertussis toxin-induced histamine sensitisation, based on the pertussis toxin-induced decrease in blood pressure in rats (Vleeming et al., 2000b) and the pertussis toxin-induced enhancement of the histamine-induced decrease in blood pressure in rats (Vleeming et al., 2000a). Because it was observed that in vivo pertussis toxin treatment affects contraction and not relaxation properties of isolated small mesenteric resistance arteries, we now hypothesise that not (a) vasodilatation-inducing signal transduction pathway(s) is (are) involved in pertussis toxin-induced histamine sensitisation, but that contraction-inducing receptors and/or their signal transduction pathway(s) are involved in pertussis toxin-induced histamine sensitisation.

In conclusion, no effect of in vivo pertussis toxin treatment on contraction or relaxation properties of isolated mouse trachea was found. In order to develop a suitable in vitro model based on the principle of the histamine sensitisation test, an in vivo effect of pertussis toxin must be identified, which can be translated into an in vitro model. Hence, it is believed that mouse tracheal smooth muscle and some of its relevant receptor systems are not good candidates for an in vitro alternative. However, in vivo pertussis toxin treatment reduced the contractile properties of isolated small mesenteric resistance arteries. We suggest that vasoconstriction-regulating mechanisms may be involved in pertussis toxin-induced histamine sensitisation. This could provide a lead for further development of an in vitro alternative for the histamine sensitisation test.

Acknowledgements

This study was financially supported by the Institutional Centre for Alternatives to Animal Testing. We thank Ineke van Straaten-Van de Kapelle who determined the biological activity of the batches of pertussis toxin.

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