No effect of pertussis toxin on peripheral prejunctional α_2 -adrenoceptor-mediated responses and on endothelium-dependent relaxations in the rat

J.R. Docherty

Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland

- 1 We have investigated the effects of pertussis toxin treatment on a variety of peripheral tissues in the rat.
- 2 Incubation with pertussis toxin ($1 \mu\text{g ml}^{-1}$) *in vitro* failed to alter the negative inotropic actions of acetylcholine in rat left atria.
- 3 Pretreatment with pertussis toxin ($6 \mu\text{g kg}^{-1}$, i.v., 3–4 days) abolished the negative inotropic actions of acetylcholine in rat left atria.
- 4 Pretreatment with pertussis toxin ($40 \mu\text{g kg}^{-1}$, i.v., 3–4 days) failed to alter the prejunctional inhibitory actions of the α_2 -adrenoceptor agonist xylazine, either in terms of the isometric contraction to a single stimulus in rat vas deferens or in terms of stimulation-evoked overflow of tritium in atria pre-incubated with [^3H]-noradrenaline.
- 5 Pretreatment with pertussis toxin ($6 \mu\text{g kg}^{-1}$, i.v., 3–4 days) failed to affect, and pertussis toxin ($40 \mu\text{g kg}^{-1}$, i.v., 3–4 days) potentiated endothelium-dependent relaxations of rat aorta to histamine and acetylcholine.
- 6 It seems unlikely that peripheral prejunctional actions of α_2 -adrenoceptor agonists or endothelium-dependent relaxations of rat aorta involve pertussis toxin-sensitive G proteins.

Introduction

Pertussis toxin catalyses the adenosine 5'-diphosphate (ADP)-ribosylation of the guanine-nucleotide binding regulatory proteins G_0 and G_i which are linked to several responses including inhibition of adenylate cyclase and opening of K^+ channels (Murayama & Ui, 1983; Dolphin, 1987). Various responses have been shown to be attenuated by pertussis toxin, including α_2 -adrenoceptor-mediated vasoconstriction in vascular smooth muscle (Boyer *et al.*, 1983), α_2 -adrenoceptor-mediated inhibition of noradrenaline release in brain slices (Allgaier *et al.*, 1985) but not in peripheral nerves (Musgrave *et al.*, 1987; Docherty, 1988), endothelium-dependent relaxations of guinea-pig pulmonary artery (Weinheimer & Osswald, 1989) and pig coronary arteries (Flavahan *et al.*, 1989). In the above studies, tissues were either incubated *in vitro* with pertussis toxin, or pretreated with pertussis toxin *in vivo*.

The present study sets out to compare pertussis toxin incubation with pretreatment and to examine the effects of pertussis toxin in 3 systems: negative inotropic actions of acetylcholine (ACh) on rat left atrium, peripheral prejunctional α_2 -adrenoceptors of rat vas deferens and right atrium, endothelium-dependent relaxations of rat aorta.

Some of these results have been presented in abstract form (Docherty, 1990).

Methods

Male Wistar rats (200–250 g) were obtained from Biolabs, Ballina, Ireland, and tissues from untreated, vehicle-treated or pertussis toxin-treated animals were employed as outlined below.

Rat left atrium

Left atria were attached to myograph transducers, under a resting isometric tension of 1 g, in organ baths at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl 119, NaHCO_3 25, D-glucose 11.1, KCl 4.7, CaCl_2 2.5,

KH_2PO_4 1.2, MgSO_4 1.0. Tissues were placed between platinum electrodes and stimulated continuously at 1 Hz (0.5 ms pulses, supramaximal voltage) to produce isometric contractions (inotropic response). When consistent isometric contractions had been obtained, ACh was administered cumulatively in 1 log unit increments beginning with $0.1 \mu\text{M}$.

Rat vas deferens

Prostatic and epididymal portions of rat vas deferens were placed between platinum electrodes and attached to myograph transducers, under a resting isometric tension of 1 g, in organ baths at 37°C in Krebs-Henseleit solution of the same composition as outlined above. Tissues were stimulated with a single stimulus (0.5 ms pulses, supramaximal voltage) every 5 min, and when consistent isometric contractions had been obtained, xylazine or dopamine was administered cumulatively in 0.5 log unit increments and a response to a single stimulus was obtained in the presence of each agonist concentration. Experiments in epididymal portions were carried out in the presence of the calcium entry blocker nifedipine ($10 \mu\text{M}$) to eliminate postjunctional actions of agonists (see Docherty, 1984).

Rat aorta

Aortic rings of approximately 3–5 mm in length were attached to myograph transducers under 1 g tension in organ baths at 37°C in Krebs-Henseleit solution of the same composition as outlined above. Tissues were contracted with KCl 40 mM, exposed to ACh $100 \mu\text{M}$, and washed. Bathing fluid was then changed every 15 min for the next 60 min. Tissues were again contracted with KCl 40 mM, and when the contraction had reached a maximum, ACh or histamine was administered cumulatively in 1 log unit increments beginning with $0.1 \mu\text{M}$ (ACh) or $1 \mu\text{M}$ (histamine). Tissues were then washed, bathing fluid was changed every 15 min for the next 60 min, KCl 40 mM was added and tissues which had received ACh now received histamine, and vice versa. In other experiments, a cumulative concentration contractile response curve to phenylephrine was obtained. In some experiments, the endothelium was deliberately removed by gentle rubbing.

Rat right atrium

Isolated right atria were preincubated for 1 h in 1 ml medium containing [^3H]-noradrenaline ($0.5\ \mu\text{M}$, specific activity $39\ \text{Ci mmol}^{-1}$) then placed in organ baths under approximately 1 g tension and superfused with [^3H]-noradrenaline-free Krebs-Henseleit solution of the same composition as listed above but containing (mM): ascorbic acid 0.28, tetrasodium EDTA 0.03, corticosterone 0.03, propranolol 0.001. Additionally, following the end of pre-incubation, cocaine ($3\ \mu\text{M}$) was added. Tissues were placed between platinum electrodes in organ baths, and superfused at a rate of $2\ \text{ml min}^{-1}$ at 37°C .

In all experiments, tissues were stimulated four times ($\text{S}_0\text{--}\text{S}_3$) for 3 min at a frequency of 2 Hz at intervals of 27 min, beginning after 2 h of superfusion.

Xylazine or distilled water vehicle was added to the superfusion stream at a rate of $50\ \mu\text{l min}^{-1}$, in 2 cumulative concentrations beginning 12 min before S_2 . Effluent samples were collected in 6 ml aliquots beginning 6 min before S_1 , and at the end of the experiment tissues were made soluble in 1 ml of tissue solubiliser. A volume of 1 ml of superfusate or dissolved tissue was added to 9 ml of liquid scintillation solution (Liquiscint) and counted in a liquid scintillation counter. Basal outflow was calculated from the sample obtained immediately before each stimulation period and expressed as a percentage of tissue tritium released per min. The stimulation-evoked overflow of tritium was calculated by subtraction of the basal outflow, and was expressed as a percentage rate (i.e. the evoked overflow during a given stimulation period was expressed as a percentage of the tritium content of the tissue at the onset of that stimulation period).

Pertussis toxin treatments

In some experiments, left atria were incubated for 2 h with pertussis toxin $1\ \mu\text{g ml}^{-1}$ or with vehicle before beginning concentration-response curves to ACh.

In most experiments, rats were anaesthetised with ether and pertussis toxin ($1\text{--}40\ \mu\text{g kg}^{-1}$) or vehicle was injected in a volume of 1 ml into the jugular vein. Experiments on isolated tissues were carried out 3–4 days after injection.

Drugs

Acetylcholine chloride (Sigma, Poole, U.K.); cocaine hydrochloride (Sigma); corticosterone (Sigma); dopamine hydrochloride (Sigma); histamine hydrochloride (Sigma); pertussis toxin (Peninsula Labs, St. Helens, U.K.); phenylephrine hydrochloride (Sigma); propranolol hydrochloride (Sigma); xylazine hydrochloride (gift: Bayer, Ireland).

Drugs were dissolved in distilled water with the exception of corticosterone (100% ethanol).

Statistics

Values are geometric mean and 95% confidence limits, or arithmetic mean $\pm$ s.e.mean. Differences between groups were compared by Student's *t* test for unpaired data.

Results

Incubation with pertussis toxin

Left atrium In left atria incubated with vehicle, ACh produced a concentration-dependent inhibition of contractions evoked by 1 Hz stimulation (negative inotropic action of ACh). Incubation with pertussis toxin $1\ \mu\text{g ml}^{-1}$ for 2 h failed to alter the negative inotropic actions of ACh (Figure 1a).

Aortic rings Incubation with pertussis toxin $1\ \mu\text{g ml}^{-1}$ for 2 h failed to alter the relaxation to histamine in KCl (40 mM)-con-

Figure 1 Effects of pertussis toxin on the inhibition by acetylcholine (ACh) of isometric contractions (inotropic response) evoked by 1 Hz continuous electrical stimulation in rat left atria. In (a) tissues were incubated for 1 h with pertussis toxin ($1\ \mu\text{g ml}^{-1}$) (Δ) or vehicle ($\bullet$). In (b) animals were pretreated with pertussis toxin $1\ (\Delta)$, $6\ (\square)$ or $40\ (\blacksquare)\ \mu\text{g kg}^{-1}$, or with vehicle ($\bullet$), intravenously 4 days before experimentation. Vertical bars represent s.e.mean from at least 4 experiments.

tracted tissue as compared with tissues incubated with vehicle (Table 1).

In all subsequent experiments, tissues were obtained from animals injected with pertussis toxin or vehicle intravenously 3–4 days previously.

Pertussis toxin pretreatment

Left atrium In left atria, pretreatment with pertussis toxin (1 , 6 or $40\ \mu\text{g kg}^{-1}$, i.v.) failed to alter the contraction evoked by 1 Hz stimulation (responses of $0.15 \pm 0.09\ \text{g}$, $n = 4$; $0.14 \pm 0.03\ \text{g}$, $n = 5$; $0.22 \pm 0.08\ \text{g}$, $n = 4$; respectively), as compared with vehicle controls ($0.21 \pm 0.05\ \text{g}$, $n = 8$). Pretreatment with pertussis toxin ($1\ \mu\text{g kg}^{-1}$, i.v.) caused a small but significant attenuation of the negative inotropic response to ACh ($100\ \mu\text{M}$) (Students' *t* test: $P < 0.05$), and pertussis toxin (6 and $40\ \mu\text{g kg}^{-1}$, i.v.) abolished the actions of ACh (Figure 1b).

Rat vas deferens

In prostatic portions of rat vas deferens, single pulse stimulation produced an isometric contraction of $2.06 \pm 0.32\ \text{g}$ ($n = 4$) in tissues from vehicle-treated animals, and $2.12 \pm 0.14\ \text{g}$ ($n = 4$) in tissues from animals pretreated with

Table 1 Maximum relaxations to histamine and acetylcholine (ACh) in KCl (40 mM)-contracted aortic rings

	Histamine	ACh
a Incubation (1 h)		
Pertussis toxin ($1\ \mu\text{g ml}^{-1}$)	$10.1 \pm 3.7\%$ ($n = 4$)	/
Vehicle	$10.8 \pm 6.3\%$ ($n = 4$)	/
b Pretreatment (3–4 days)		
Pertussis toxin ($6\ \mu\text{g kg}^{-1}$)	$14.5 \pm 3.7\%$ ($n = 4$)	$26.6 \pm 1.5\%$ ($n = 4$)
Pertussis toxin ($40\ \mu\text{g kg}^{-1}$)	$46.5 \pm 9.6\%*$ ($n = 4$)	$52.8 \pm 9.3\%$ ($n = 4$)
Vehicle	$18.0 \pm 1.3\%$ ($n = 6$)	$34.4 \pm 4.5\%$ ($n = 6$)

Relaxations are expressed as a percentage of the contraction to KCl (mean $\pm$ s.e. from at least 4 experiments). In (a), tissues were incubated for 1 h with pertussis toxin ($1\ \mu\text{g ml}^{-1}$) or with vehicle. In (b), animals were pretreated with pertussis toxin (6 or $40\ \mu\text{g kg}^{-1}$, i.v.) or vehicle 3–4 days before experimentation. Asterisks denote response significantly different from response in vehicle tissues (Student's *t* test. * $P < 0.05$).

Figure 2 Inhibition by xylazine of the isometric contraction to a single electrical stimulus in prostatic portions of rat vas deferens. (●) Represent responses from vehicle-pretreated animals and (■) represent responses from pertussis toxin ($40\text{ }\mu\text{g kg}^{-1}$, i.v., 3–4 days) pretreated animals. Vertical bars represent s.e.mean from at least 3 experiments.

pertussis toxin ($40\text{ }\mu\text{g kg}^{-1}$, i.v., 3–4 days) (no significant difference). Pertussis toxin did not significantly alter the inhibition by xylazine of stimulation-evoked contractions (Figure 2 and Table 2).

In epididymal portions of rat vas deferens in the presence of nifedipine ($10\text{ }\mu\text{M}$), single pulse stimulation produced an isometric contraction of $0.92 \pm 0.38\text{ g}$ ($n = 3$) in tissues from vehicle-treated animals, and $1.51 \pm 0.24\text{ g}$ ($n = 3$) in tissues from animals pretreated with pertussis toxin ($40\text{ }\mu\text{g kg}^{-1}$ i.v., 3–4 days) (no significant difference). Dopamine ($1\text{--}300\text{ }\mu\text{M}$) produced concentration-dependent inhibitions of the stimulation-evoked contraction, but there were no significant differences between tissues from vehicle-treated and pertussis toxin-treated animals in terms of the potency of dopamine (Table 2).

Rat aorta

In aortic rings, KCl (40 mM) produced contractions of $0.82 \pm 0.07\text{ g}$ ($n = 6$), $0.69 \pm 0.16\text{ g}$ ($n = 4$) and $0.63 \pm 0.03\text{ g}$ ($n = 4$) in tissues from vehicle-treated animals and animals pretreated with pertussis toxin $6\text{ }\mu\text{g kg}^{-1}$ and $40\text{ }\mu\text{g kg}^{-1}$,

Figure 3 Relaxations to (a) acetylcholine (ACh) and (b) histamine in aortic rings contracted with KCl 40 mM from vehicle pretreated (●), or pertussis toxin $6\text{ }\mu\text{g kg}^{-1}$ (□), or $40\text{ }\mu\text{g kg}^{-1}$ (i.v., 3–4 days)-pretreated rats. Vertical bars represent s.e.mean from at least 4 experiments.

respectively (no significant differences). The maximum contraction to phenylephrine was $1.13 \pm 0.06\text{ g}$ ($n = 3$) and $0.82 \pm 0.05\text{ g}$ ($n = 3$) in tissues from vehicle-treated animals and animals pretreated with pertussis toxin ($40\text{ }\mu\text{g kg}^{-1}$), respectively ($P < 0.05$). Pertussis toxin ($6\text{ }\mu\text{g kg}^{-1}$) did not significantly alter the relaxations to histamine ($1\text{--}300\text{ }\mu\text{M}$) or ACh ($0.1\text{--}100\text{ }\mu\text{M}$) in KCl (40 mM) contracted tissues (Figure 3). However, pertussis toxin ($40\text{ }\mu\text{g kg}^{-1}$) significantly increased the maximum relaxation to histamine ($P < 0.05$) and significantly increased the potency of ACh ($P < 0.05$) (Figure 3; Tables 1 and 2).

In endothelium-denuded preparations contracted with KCl, ACh did not produce relaxations and histamine ($100\text{ }\mu\text{M}$) produced contractions of $0.07 \pm 0.01\text{ g}$ ($n = 3$).

Rat right atrium

Field stimulation of rat isolated right atria at a frequency of 2 Hz for 3 min produced an evoked overflow of tritium: an S_1 of $1.05 \pm 0.18\%$ ($n = 4$) and $1.20 \pm 0.15\%$ ($n = 4$) of tissue tritium in tissues from vehicle-treated and pertussis toxin ($40\text{ }\mu\text{g kg}^{-1}$)-treated animals, respectively (no significant difference). Pertussis toxin failed to alter the inhibition by xylazine of stimulation-evoked release of tritium (Figure 4).

Discussion

In this study, incubation for 2 h with pertussis toxin ($1\text{ }\mu\text{g ml}^{-1}$) failed to affect negative inotropic responses of rat

Table 2 Potency of xylazine or dopamine in rat vas deferens expressed as an IC_{50} (concentration producing 50% inhibition of the isometric contraction to a single stimulus) and potency of histamine and acetylcholine (ACh) in rat aorta expressed as an EC_{50} (concentration producing 50% of maximum relaxation in KCl-contracted tissues)

	Vehicle	Pertussis toxin	
		$6\text{ }\mu\text{g kg}^{-1}$	$40\text{ }\mu\text{g kg}^{-1}$
Vas deferens			
Xylazine IC_{50} (nM)	98 (44–220)	/	132 (44–398)
Dopamine IC_{50} (μM)	102.3 (40.7–257)	/	66.1 (10.7–407)
Rat aorta			
Histamine EC_{50} (μM)	12.0 (9.3–15.5)	13.2 (8.7–20.0)	4.8 (0.91–25.1)
ACh EC_{50} (μM)	0.59 (0.34–1.02)	1.05 (0.44–2.5)	0.17* (0.035–0.87)

Tissues were pretreated with pertussis toxin (6 or $40\text{ }\mu\text{g kg}^{-1}$, i.v.) or vehicle 3–4 days before experimentation. Values are mean and 95% confidence limits from at least 3 experiments. Asterisks denote responses significantly different from response in vehicle-treated tissues (Student's t test. * $P < 0.05$).

Figure 4 Effects of xylazine on 2 Hz stimulation-evoked overflow of tritium in atria from vehicle (●)- or pertussis toxin $40\text{ }\mu\text{g kg}^{-1}$ (i.v., 3–4 days) (■)-pretreated rats. Vertical bars represent s.e.mean from 4 experiments.

left atrium to acetylcholine, and in a previous study even 16 h incubation with pertussis toxin ($1 \mu\text{g ml}^{-1}$) produced only a limited reduction of the negative inotropic response of mouse atria to carbachol (Musgrave *et al.*, 1987). Although other authors found that incubation with pertussis toxin 200 ng ml^{-1} for 1 h (Flavahan *et al.*, 1989) or $1 \mu\text{g ml}^{-1}$ for 2 h (Weinheimer & Osswald, 1989) inhibited endothelium-dependent relaxations to 5-hydroxytryptamine or histamine, these authors did not examine the effects of pertussis toxin treatment on negative inotropic responses of atria to muscarinic agonists. In contrast, pretreatment with pertussis toxin ($6 \mu\text{g kg}^{-1}$) intravenously 3–4 days before the experimental day abolished the negative inotropic actions of ACh, and even pertussis toxin $1 \mu\text{g kg}^{-1}$ produced some attenuation. Similarly, pretreatment with pertussis toxin ($1.5 \mu\text{g}$) intravenously 4 days before the experimental day abolished the negative inotropic action of carbachol in mouse atria (Musgrave *et al.*, 1987), and pretreatment with pertussis toxin ($25 \mu\text{g}$) 40 h before the experimental day abolished the negative inotropic action of carbachol in rat atria. Hence, pretreatment with pertussis toxin *in vivo* is a more effective means of inactivating the G protein, G_i or G_o (Endoh *et al.*, 1985; Dolphin, 1987), involved in the negative inotropic actions of muscarinic agonists than incubation with the toxin *in vitro*. Although this does not necessarily mean that G_i or G_o in other tissues is blocked by the pretreatment, it should still imply that G proteins in other tissues are more likely to be blocked by toxin pretreatment than by incubation with toxin.

Pretreatment with pertussis toxin ($40 \mu\text{g kg}^{-1}$ i.v. 3–4 days), a dose 6 times higher than necessary to abolish, and 40 times higher than necessary to reduce, negative inotropic actions of ACh in atria, failed to influence the prejunctional inhibitory actions of the α_2 -adrenoceptor agonist xylazine, either in terms of the inhibition of isometric contractions in prostatic portions of rat vas deferens or in terms of the inhibition of stimulation-evoked release of tritium in rat right atria. Likewise, inhibitory responses to dopamine (present results) and to 5-hydroxytryptamine (Borton & Docherty, unpublished) are unaffected by pertussis toxin in vas deferens. This is in agreement with our previous findings that pretreatment with pertussis toxin (50 – $200 \mu\text{g kg}^{-1}$, i.p. 18 h) failed to alter the prejunctional inhibitory actions of xylazine in pithed rat heart and in epididymal portions of rat vas deferens (Docherty, 1988). Similar findings have been obtained for mouse atria (Musgrave *et al.*, 1987). However, other authors have shown that incubation of brain slices with pertussis toxin for 6 h produced a relatively small reduction in the prejunctional actions of α_2 -adrenoceptor agonists and antagonists (Allgaier *et al.*, 1985). However, this incubation with pertussis toxin significantly increased the stimulation-evoked release of noradrenaline (Allgaier *et al.*, 1985; see also Allgaier *et al.*, 1987), although such an increase was not found in the present study of rat atria pretreated with pertussis toxin.

Pertussis toxin $6 \mu\text{g kg}^{-1}$ (i.v., 3–4 days) failed to affect the endothelium-dependent relaxations to histamine or ACh in rat aorta precontracted with KCl and pertussis toxin $40 \mu\text{g kg}^{-1}$ actually potentiated the relaxant actions of these agents. The reason for this is unclear, although the maximum relaxation to a vasodilator in rat aorta can be altered by varying the concentration of contractile agent (see Sawyer & Docherty, 1987), so alterations in the contractile response to KCl might explain such an effect. Admittedly, the contractile response to KCl was not significantly reduced by pertussis toxin ($40 \mu\text{g kg}^{-1}$) even though the maximum contraction to phenylephrine was significantly reduced. Other authors have shown that pertussis toxin pretreatment reduced the maximum contraction to α_1 -adrenoceptor agonists in rabbit pulmonary artery (Liebau *et al.*, 1989).

Some authors have demonstrated that incubation for 1 h with pertussis toxin (100 ng ml^{-1}) virtually abolished endothelium-dependent relaxations produced by 5-hydroxytryptamine in pig coronary artery (Flavahan *et al.*, 1989), and that incubation for 2 h with pertussis toxin ($1 \mu\text{g ml}^{-1}$) inhibited endothelium-dependent relaxations to histamine in guinea-pig pulmonary artery (Weinheimer & Osswald, 1989) without affecting endothelium-dependent relaxations to the calcium ionophore A23187 in either tissue. Although these differences from the present results may reflect tissue and species differences in endothelium-dependent relaxations, two points should be noted. In the above studies, the effectiveness of the pertussis toxin incubation was not independently assessed in another test system, such as attenuation of negative inotropic responses in atria. Secondly, in both studies the pertussis toxin incubation attenuated responses to agonists (5-hydroxytryptamine, histamine) which produced maximum relaxations of less than 100% of the response to the contractile agent, whereas responses resistant to pertussis toxin involved agonists (calcium ionophore) which produced maximum relaxations of 100% or more of the response to the contractile agent (Flavahan *et al.*, 1989; Weinheimer & Osswald, 1989). Hence, apparent resistance to pertussis toxin may reflect the maximal nature of the relaxation to some agents, analogous to the effects of ageing on vascular relaxations in rat aorta, where age-related alterations may be difficult to demonstrate in the response to agents producing relaxations of a magnitude sufficient to abolish the KCl contraction in young animals (Sawyer & Docherty, 1987).

Two questions remain to be considered concerning pertussis toxin treatments. Firstly, is the effect of pretreatment *in vivo* different from the effect of incubation *in vitro*? In our hands, *in vitro* incubation by use of a methodology similar to that of Flavahan *et al.* (1989) or Weinheimer & Osswald (1989) failed to produce any discernible effect, although the source of pertussis toxin differed between laboratories. Secondly, is there a problem of access for pertussis toxin to cells other than atrial cells? This seems unlikely since neuronal responses to opioids (Werling *et al.*, 1989) and dopamine (Bean *et al.*, 1988) in brain slices are attenuated by pertussis toxin pretreatment. Although the possibility remains that peripheral neurones differ from central neurones in the ability of pertussis toxin to penetrate, especially since responses to dopamine and 5-hydroxytryptamine-receptor agonists as well as α_2 -adrenoceptor agonists proved to be resistant to pertussis toxin in peripheral nerves. The evidence for a lack of involvement of G_i or G_o in these responses would have been stronger if a neuronal response had been found which was susceptible to pertussis toxin in vas deferens or atria. However, some but not all effects of adenosine at A_1 -receptors in the rat hippocampus are resistant to pertussis toxin (Fredholm *et al.*, 1989), so that even some central inhibitory receptors mediate responses resistant to pertussis toxin. It seems unlikely that peripheral neurones have a large excess of G_i or G_o , since even doses of pertussis toxin 6 times higher than necessary to abolish, and 40 times higher than necessary to attenuate, negative inotropic responses of atria were totally ineffective. However, a combination of reduced access for pertussis toxin and a large excess of G_i or G_o could explain resistance to the toxin.

In conclusion, although we cannot categorically state that these responses do not involve G_i or G_o , it seems unlikely that endothelium-dependent relaxations of rat aorta to ACh or histamine and prejunctional inhibitory actions of α_2 -adrenoceptor agonists in rat vas deferens and atria involve pertussis toxin-sensitive G proteins, G_i or G_o .

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