Effects of Pertussis Toxin on Intraocular Pressure and Adenylate Cyclase Activity of Ciliary Processes in Rabbits

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Abstract. The effects of pertussis toxin on the intraocular pressure (IOP) lowering effect of clonidine and isoproterenol as well as on the inhibitory effects of clonidine and neuropeptide Y on adenylate cyclase activity of ciliary processes were studied in albino rabbits. I.v. administered pertussis toxin elicited transient changes in IOP which, however, returned to control values during 2–3 days. In the following days the IOP lowering effect of the α_2-adrenergic agonist clonidine was abolished and that of the nonselective β-adrenergic agonist isoproterenol was attenuated. At the same time, the inhibitory effects of clonidine and neuropeptide Y on basal as well as stimulated adenylate cyclase activities in homogenates of ciliary processes were grossly diminished. The effects of pertussis toxin on the IOP lowering action of adrenergic agonists and on the inhibitory action of clonidine and neuropeptide Y on adenylate cyclase activity were ascribed to an impairment of the function of a G protein in ciliary processes, probably G_i protein. It is suggested that the decrease of IOP induced by clonidine is due to inhibition of adenylate cyclase.

Key words: Adenylate cyclase — Adrenergic receptors — Ciliary processes — G protein — Intraocular pressure (IOP) — Pertussis toxin

Introduction

Several authors have shown that stimulation of α_2-adrenergic receptors can inhibit the basal and drug-stimulated production of cyclic AMP as well as adenylate cyclase activity in rabbit ciliary processes (Mittag and Tormay 1985; Bausher et al. 1987; 1989; Kintz et al. 1988; Čepelík and Hynie 1990a). It is primarily an α_2-adrenergic agonist, clonidine, which reveals this inhibitory effect. Recently, we have shown

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that neuropeptide Y (NPY) also inhibits basal and/or drug-stimulated adenylate cyclase activity in homogenates from rabbit ciliary processes (Čepelík and Hynie 1990b).

The inhibition of adenylate cyclase activity in ciliary processes may contribute to the well known ocular hypotensive effect of clonidine (Allen and Langham 1976; Murray and Leopold 1985). However, it is hard to reconcile this assumption with the widely accepted view that stimulation of adenylate cyclase activity in ciliary processes is the very cause of the decrease of intraocular pressure (IOP) elicited by agents that increase cyclic AMP level in this tissue (Sears and Mead 1983; Sears et al. 1984; Mittag et al. 1987).

The inhibitory effects of α_2-adrenergic agonists on adenylate cyclase activity have been shown to be mediated via G_i regulatory protein in several tissues. Furthermore, it has been reported that this inhibitory protein can be ADP-ribosylated by pertussis toxin both *in vitro* and *in vivo* and that the modified protein is unable to transfer the signal from stimulated inhibitory receptors to the catalytic unit of adenylate cyclase (Ui 1984). It follows that, after pertussis toxin pretreatment, adenylate cyclase becomes insensitive to regulatory effects of inhibitory receptors. Thus, one of the most direct proofs of the involvement of the G_i protein in the process under study would be a reduction, after pertussis toxin pretreatment, of the effects of those drugs which inhibit adenylate cyclase via inhibitory receptors.

Having in mind all these facts, we decided to study the effects of pertussis toxin administered *in vivo* on the effects of several drugs on adenylate cyclase in ciliary processes and on intraocular pressure in rabbits. Preliminary accounts of these results have been presented earlier (Hynie and Čepelík 1990).

Materials and Methods

Animals. New Zealand albino rabbits (2.5–3.5 kg) were used throughout the study. All procedures involving animals conformed with the Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80–23).

Intraocular pressure measurement. IOP was measured in conscious animals after topical anaesthesia with 50 μl of 0.25% tetracaine using an applanation pneumatonograph (Digilab Inc., Cambridge, MA.). Pupil diameter was estimated using a transparent millimeter ruler. Drugs tested for the effects on IOP were dissolved in distilled water and administered topically in 50 μl volume into the conjunctival sac of the animal. In the contralateral conjunctival sac, 50 μl of distilled water was applied.

Adenylate cyclase assay. The preparation of homogenates from ciliary processes was performed as described previously (Čepelík and Hynie 1990a). Protein content in homogenates was estimated by utilizing Folin-phenol reagent (Lowry et al. 1951). Adenylate cyclase assay was performed using ^{32}P-α-ATP as the substrate (Hynie 1990). Radioactive cyclic AMP formed was separated using aluminum column chromatography as described earlier (Čepelík and Hynie 1990a).

Pertussis toxin preparation. The partially purified pertussis toxin (Hynie 1986; 1990), as used in several previous studies (Hynie 1987; Tuček et al. 1987; Doležal et al. 1989), was administered into the ear vein in two successive doses. The first dose was equivalent to 5.0 μg per kg of a commercial pertussis toxin (see *Drugs*), when estimated according to the lipolytic effect on rat adipose tissue preparations *in vitro* (Hynie 1987) (data not shown). The second dose, administered 96 h later, was equivalent to 1.0 μg per kg of a commercial preparation of pertussis toxin. Heat-inactivated pertussis toxin was prepared by heating the toxin preparation for 30 min in boiling water. The ineffectiveness of pertussis toxin was confirmed by checking its lipolytic activity on rat adipose tissue preparation *in vitro*.

Statistical analysis. Data in Figures and Table 1 are presented as mean values ± S.E.M. from the number of experiments indicated as n. Results were analyzed using one-way Analysis of Variance (ANOVA) and the Newman-Keuls test was used to evaluate the significance of differences between groups of experiments. The accepted level of significance for all tests was $P < 0.05$.

Drugs. Forskolin was from Calbiochem, San Diego, USA. (-)-Isoproterenol d-bitartrate dihydrate was a product of Janssen Pharmaceutica, Belgium. Clonidine hydrochloride was generously provided by Boehringer Ingelheim, Ingelheim, FRG. Porcine neuropeptide Y (Peninsula, Belmont, USA) was a generous gift of Dr. C. Wahlestedt. Pertussis toxin was a product of List Biological Laboratorie, Inc. Campbell, USA, ^{32}P-α-ATP was prepared in our laboratory by the procedure of Symons (1977) as described by Hynie (1990). All other chemicals were commercial preparations and were used without further purification.

Results

The effects of pertussis toxin alone on IOP

Figure 1 documents both acute and prolonged changes in IOP elicited by two successive i.v. doses of pertussis toxin to rabbits. Administration of an equivalent of 5.0 μg/kg of pertussis toxin led to a remarkable increase of IOP in the first hour after its application. This was followed by a rapid return of IOP to levels even slightly below the control levels in the second hour and by a stepwise significant decrease of IOP up to a minimum 5–7 hours after administration. During subsequent three to four days, IOP returned to the control levels. A rechallenge of the animals 96 hours after the first dose by the administration of an equivalent of 1.0 μg/kg of pertussis toxin led to qualitatively similar, though substantially diminished, changes in IOP with its recovery to basal values in 2–3 days. When the smaller dose of pertussis toxin was administered as a single dose, it led to a clear-cut decrease of IOP. This decrease, however, was much less pronounced than the decrease of IOP seen after the higher dose of pertussis toxin, and again, IOP returned to control levels during 2–3 days. The administration of a heat-inactivated pertussis toxin equivalent of 5.0 μg/kg was followed by a decrease of IOP with a time-course similar to that seen after the administration of active pertussis toxin (data not shown). Clinically, all rabbits treated with intravenous pertussis toxin developed massive conjunctival and iridial hyperemia, corneal and conjunctival oedema, flare of aqueous humor and

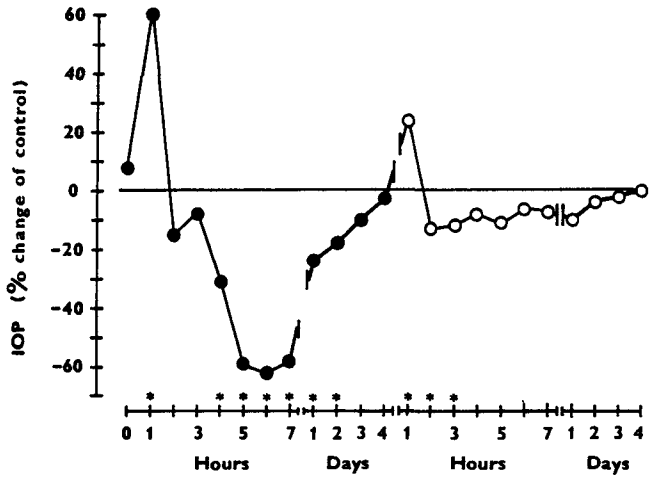

Figure 1. The effects of intravenously administered pertussis toxin on intraocular pressure (IOP) of albino rabbits. —●— Changes in IOP following the administration of pertussis toxin in a dose equivalent to 5 μg/kg at time 0. —○— Changes in IOP following a rechallenge of the animals by pertussis toxin in a dose eqivalent to 1.0 μg/kg, administered 96 h later. Two groups of rabbits were used throughout the experiment; a pertussis toxin-treated group ($n = 6$) and a control group of animals treated with a corresponding amount of physiological saline ($n = 6$). At every point of IOP measurements an average IOP for both eyes of control rabbits was taken as 100% and the results were expressed as percent change vs. controls; significant changes for $P < 0.05$ are indicated by an asterisk.

miosis within the first hours after pertussis toxin administration. These changes gradually declined and all signs and symptoms disappeared within 2 to 3 days. In further experiments, the animals were pretreated with pertussis toxin according to the two-dose protocol described above. All experiments were performed 8 to 16 days after the administration of the first dose of pertussis toxin.

The influence of pertussis toxin pretreatment on effects of IOP lowering drugs

Figures 2*A* and 2*B* compare the effects of topically applied α_2-adrenergic agonist clonidine on IOP in control rabbits and in animals pretreated with pertussis toxin. The administration of 50 μl of 0.25% clonidine to one conjunctival sac control animals elicited a marked increase of IOP in the eye within the first hour after the drug application. This was followed by a clear-cut decrease of IOP during next hours with a maximum effect at 4 h. In pertussis toxin pretreated animals this decrease of IOP was almost totally abolished though a rise of IOP elicited by clonidine remained uninfluenced (Fig. 2*A*). In control animals, only a decrease

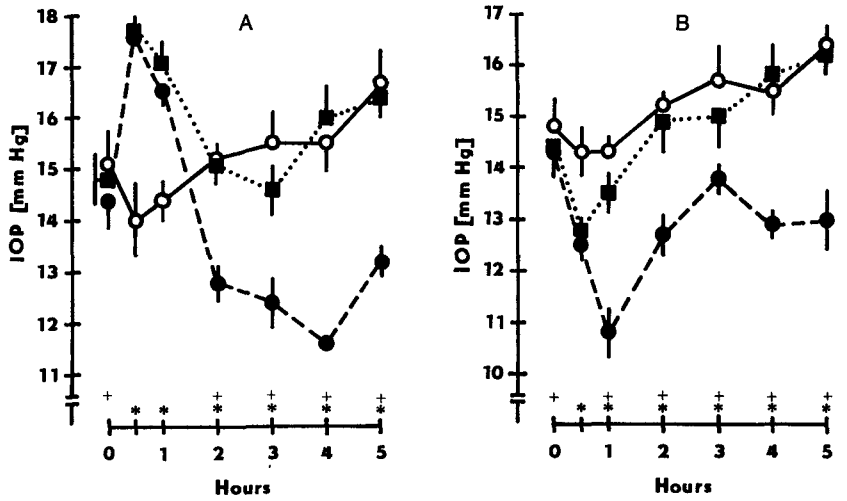

Figure 2*A*. The influence of pertussis toxin-pretreatment on IOP lowering effect of clonidine in the treated (ipsilateral) eyes. *a*) —o— IOP in the ipsilateral eyes of control (untreated by pertussis toxin) animals. Corresponding values of pertussis toxin-treated animals were not significantly different from controls and therefore, for the sake of simplicity of the Figure, they were omitted. *b*) - - -●- - - IOP in the eyes treated with 50 μl of 0.25% clonidine at time 0; pertussis toxin-untreated animals. *c*) ···■··· IOP in the eyes treated with clonidine; animals pretreated with pertussis toxin. The values shown are mean values of IOP (mm Hg) ± S.E.M. measured for six eyes for each point; * indicates statistically significant difference ($P < 0.05$) between corresponding values for groups *a*) and *b*), + indicates no statistically significant difference between corresponding values for groups *a*) and *c*). All animal groups were measured simultaneously, together with groups the results for which are shown in Fig. 2*B*.

Figure 2*B*. The influence of pertussis toxin-pretreatment on IOP lowering effect of clonidine in untreated (contralateral) eyes. *a*) —o— IOP in the contralateral eyes of control (untreated by pertussis toxin) animals. The corresponding values for pertussis toxin-treated animals were not significantly different from controls and therefore, for the sake of simplicity of the Figure, they were omitted. *b*) - - -●- - - IOP in the eyes contralateral to those treated with 50 μl of 0.25% clonidine at time 0; pertussis toxin-untreated animals. *c*) ···■··· IOP in the eyes contralateral to those treated with clonidine; animals pretreated with pertussis toxin. The values shown are mean values of IOP (mm Hg) ± S.E.M. measured for six eyes for each point; * indicates statistically significant difference ($P < 0.05$) between corresponding values for groups *a*) and *b*), + indicates no statistically significant difference between corresponding values for groups *a*) and *c*). All animal groups were measured simultaneously, together with groups the results for which are shown in Fig. 2*A*.

of IOP in the contralateral eyes was seen after clonidine application and this was again practically abolished in pertussis toxin pretreated rabbits (Fig. 2*B*).

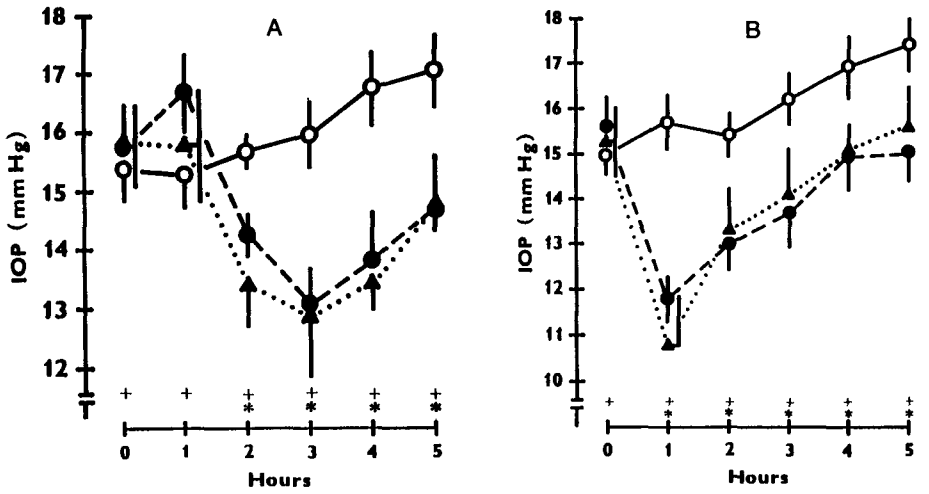

Figure 3*A*. The influence of pretreatment with heat-inactivated pertussis toxin (HIPT) on IOP lowering effect of clonidine in the treated (ipsilateral) eyes. *a*) —o— IOP in the ipsilateral eyes of control (untreated with HIPT) animals. The corresponding values for HIPT-treated animals were not significantly different from controls and therefore, for the sake of simplicity of the Figure, they were omitted. *b*) – – –●– – – IOP in the eyes treated with 50 μl of 0.25% clonidine at time 0; HIPT-untreated animals. *c*) ···▲··· IOP in the eyes treated with clonidine; animals pretreated with HIPT. The values shown are mean values of IOP (mm Hg) ± S.E.M. measured for six eyes for each point; * indicates statistically significant difference ($P < 0.05$) between corresponding values for groups *a*) and *b*), + indicates no statistically significant difference between corresponding values for groups *b*) and *c*). All animal groups were measured simultaneously, together with groups the results for which are shown in Fig. 3*A*.

Figure 3*B*. The influence of pretreatment with heat-inactivated pertussis toxin (HIPT) on lowering effect of clonidine in the untreated (contralateral) eyes. *a*) —o— IOP in the contralateral eyes of control (untreated by HIPT) animals. The corresponding values for HIPT treated animals were not significantly different from controls and therefore, for the sake of simplicity of the Figure, they were omitted. *b*) – –●– – IOP in the eyes contralateral to those treated with 50 ml of 0.25% clonidine at time 0; HIPT-untreated animals. *c*) ···▲··· IOP in the eyes contralateral to those treated with clonidine; animals pretreated with HIPT. The values shown are mean values of IOP (mm Hg) ± S.E.M. measured for six eyes for each point; * indicates statistically significant difference ($P < 0.05$) between corresponding values for groups *a*) and *b*), + indicates no statistically significant difference between corresponding values for groups *b*) and *c*). All animal groups were measured simultaneously, together with groups the results for which are shown in Fig. 3*A*.

The influence on the ocular hypotensive effect of clonidine of pretreatment of rabbits with heat-inactivated pertussis toxin is shown in Figs. 3*A* and 3*B*. The effect of clonidine was not abolished by the inactivated toxin in either treated or untreated eyes.

In addition to IOP measurements, we estimated the diameter of pupils. In the first phase of its effect, clonidine elicited mydriasis in ipsilateral eyes, which was not influenced by either active or inactivated pertussis toxin. The diameter of pupils in the contralateral eyes was unaffected by clonidine both in normal and pertussis toxin pretreated rabbits (data not shown).

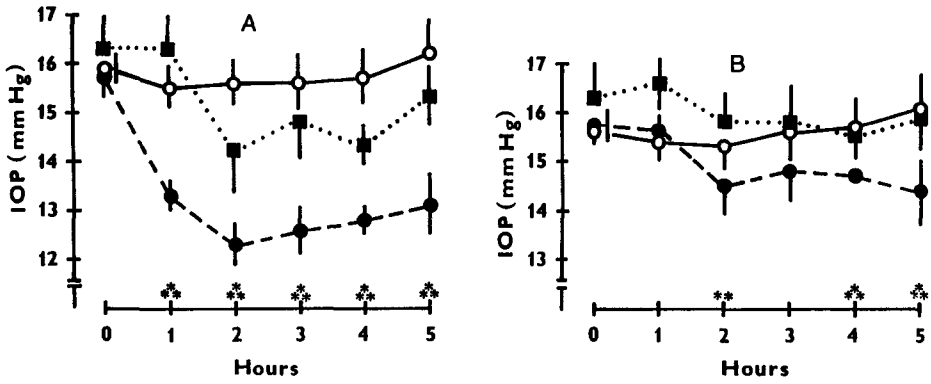

Figure 4*A*. The influence of pertussis toxin-pretreatment on IOP lowering effect of isoproterenol in the treated (ipsilateral) eyes. *a*) —o— IOP in the ipsilateral eyes of control (untreated by pertussis toxin) animals. The corresponding values of pertussis toxin-treated animals were not significantly different from controls and therefore, for the sake of simplicity of the Figure, they were omitted. *b*) – –●– – IOP in the eyes treated with 50 μl of 1% isoproterenol at time 0; pertussis toxin-untreated animals. *c*) · · ·■· · · IOP in the eyes treated with isoproterenol; animals pretreated with pertussis toxin. The values shown are mean values of IOP (mm Hg) ± S.E.M. measured for six eyes for each point; * indicates statistically significant difference ($P < 0.05$) between corresponding values for groups *a*) and *b*), ** indicate statistically significant difference between corresponding values for groups *b*) and *c*). All animal groups were measured simultaneously, together with groups the results for which are shown in Fig. 4*B*.

Figure 4*B*. The influence of pertussis toxin-pretreatment on IOP lowering effect of isoproterenol in the untreated (contralateral) eyes. *a*) —o— IOP in the contralateral eyes of control (untreated by pertussis toxin) animals. The corresponding values for pertussis toxin-treated animals were not significantly different from controls and therefore, for the sake of simplicity of the Figure, they were omitted. *b*) – –●– – IOP in the eyes contralateral to those treated with 50 μl of 1% isoproterenol at time 0; pertussis toxin-untreated animals. *c*) · · ·■· · · IOP in the eyes contralateral to those treated with isoproterenol; animals pretreated with pertussis toxin. The values shown are mean values of IOP (mm Hg) ± S.E.M. measured for six eyes for each point; * indicates statistically significant difference ($P < 0.05$) between corresponding values for groups *a*) and *b*). All animal groups were measured simultaneously, together with groups the results for which are shown in Fig. 4*A*.

The influence of pertussis toxin pretreatment on changes in IOP elicited by a topical administration of a β-adrenergic agonist isoproterenol is shown in Figs. 4*A* and 4*B*. In control animals, there was a clear-cut decrease of IOP in the eyes treated with 1% isoproterenol. In the contralateral eyes the decrease of IOP was substantially less marked than that in the ipsilateral eyes, and it was statistically insignificant. In rabbits pretreated with pertussis toxin, the decrease of IOP elicited by isoproterenol was remarkably reduced but not totally abolished in ipsilateral eyes; in the contralateral eyes the effect of isoproterenol was completely abolished.

Table 1. Reduction of the inhibitory effect of clonidine and neuropeptide Y on adenylate cyclase activity in rabbit ciliary processes from pertussis toxin (PT) untreated (controls) and treated rabbits. Data are expressed as percentage inhibition of respective adenylate cyclase activity (taken for 100%); absolute values (in pmoles/mg protein per 5 min ± S.E.M.) for pertussis toxin untreated and treated groups were as follows: basal = 82.3±5.2 and 85.2±4.5; 3 μmol/l isoproterenol-stimulated = 210.6±10.3 and 215.2±12.8; 10 μmol/l forskolin-stimulated = 690.5 ± 21.9 and 680.7 ± 42.0, respectively. Data represent pooled values from 6 experiments performed in triplicate. Inter-group differences were evaluated by Student's *t*-test for $P < 0.05$.

Group	Inhibition of adenylate cyclase activity			
	Clonidine, 10 μmol/l		Neuropeptide Y, 1 μmol/l	
	Controls	PT treated	Controls	PT treated
	Per cent inhibition			
Basal	27.5 ± 3.0[a]	12.5 ± 5.0[b]	16.5 ± 3.5[a]	4.0 ± 1.5[b]
Isoproterenol-stimulated (3 μmol/l)	30.7 ± 2.5[a]	15.2 ± 2.1[b]	20.0 ± 2.1[a]	10.1 ± 2.5[b]
Forskolin-stimulated (10 μmol/l)	27.6 ± 2.7[a]	18.7 ± 2.2[b]	–	

[a]Significant ($P < 0.05$) inhibition by clonidine or neuropeptide Y in controls; [b]Significant ($P < 0.05$) reduction of the inhibitory effects of clonidine or neuropeptide Y in PT treated groups.

The influence of pertussis toxin pretreatment on adenylate cyclase activity

The next step of this study involved estimation of the effects of clonidine and neuropeptide Y (NPY) on the adenylate cyclase activity in ciliary processes removed from control or pertussis toxin pretreated rabbits. The protocol for pertussis toxin administration was the same as shown in Fig. 1. Table 1 compares the inhibitory effects of clonidine or neuropeptide Y on basal or drug-stimulated adenylate cyclase activities of ciliary processes of control and pertussis toxin pretreated rabbits.

Neither basal nor isoproterenol- or forskolin-stimulated adenylate cyclase activity from pertussis toxin pretreated animals differed significantly from adenylate cyclase activities measured for control animals. An inhibition by approx. 30% of basal as well as isoproterenol- and forskolin-stimulated adenylate cyclase activities in homogenate preparations from ciliary processes of control animals was observed in the presence of 10 μmol/l clonidine. This inhibitory effect of clonidine was distinctly reduced by 32–55% in adenylate cyclase preparations from animals pretreated with active pertussis toxin. Similarly, the inhibitory effects of neuropeptide Y on adenylate cyclase activity of ciliary processes from pertussis toxin pretreated animals was reduced by 49–76%. On the contrary, the inhibitory effects of clonidine and neuropeptide Y on adenylate cyclase activity of ciliary processes were not significantly influenced in preparations from heat-inactivated pertussis toxin pretreated rabbits (data not shown).

Discussion

The results demonstrate that pertussis toxin preparation administered to rabbits *in vivo* had remarkable two phase effects on IOP. In the first early phase pertussis toxin administration elicited a distinct though short-lived increase of IOP followed by a profound decrease of IOP lasting for several hours. Then, there was a stepwise return of IOP to pretreatment levels during 2–3 days. In the second late phase IOP *per se* was at pretreatment levels but a clear-cut attenuation or even abolition of IOP lowering effects of some adrenergic agonists was apparent. In addition to the above mentioned effects on IOP, a diminution of the inhibitory effects of α_2-adrenergic agonist clonidine and neuropeptide Y on adenylate cyclase activity of ciliary processes was also apparent during this second phase of pertussis toxin effect. To maintain sufficient effect of pertussis toxin for the prolonged time of this experiment, the toxin was applied twice as described in Materials and Methods.

The mechanism of the early changes in IOP elicited by pertussis toxin administration was not studied further and remains unclear. However, a participation of the inhibitory effects of pertussis toxin on G proteins function in this effect of pertussis toxin seems quite unlikely. This contention is supported by the finding that IOP had returned to pretreatment levels by the time when an inhibition of G protein by pertussis toxin in ciliary processes could be demonstrated. Moreover, a clear-cut inhibition of G_i and other pertussis toxin-sensitive proteins by pertussis toxin is known to take several hours, both *in vitro* and *in vivo*. Since the early changes in IOP elicited by pertussis toxin were accompanied by signs of an inflammatory reaction in the eyes, it seems quite possible that they were a manifestation of a nonspecific effect of the exogenously delivered xenoprotein. Similar reaction has been seen by others after the administration of some other bacterial toxins (Ayo 1943; Levene and Breinin 1959). This assumption is further strengthened

by the fact that our preparation of pertussis toxin was only partially purified. Of course, the possibility that the ability of pertussis toxin to act as a histamine sensitizing and lymphocytosis- promoting factor (Yajima et al. 1978) contributing to this effect cannot be ruled out. After all, however, the early changes in IOP were probably elicited by a nonspecific effect of pertussis toxin.

On the contrary, several lines of evidence indicate that the inhibition of the G_i protein or, possibly, of more types of regulatory G proteins, was the most probable cause of the ability of pertussis toxin to abolish the IOP lowering effect of the α_2-adrenergic agonist clonidine and also to attenuate the same effect of the nonselective β-adrenergic agonist isoproterenol. First, the effects of pertussis toxin on ocular hypotensive actions of these adrenergic agents were tested after IOP of the animals pretreated with pertussis toxin had returned to normal level. Thus, any unspecific effect of pertussis toxin, similar to the above mentioned one, was unlikely. Secondly, the heat-inactivated pertussis toxin did not display any influence on the IOP lowering effect of clonidine though *per se* it elicited early effects on IOP similar to those of the fully active pertussis toxin. Thus, the inhibition of IOP lowering effects of adrenergic agonists by pertussis toxin is probably a result of a specific action of this toxin. The only known specific effect of pertussis toxin is that on the function of pertussis toxin-sensitive G proteins (Ui 1984). Accordingly, our results showing the inhibition of IOP lowering effects of adrenergic agonists *in vivo* and simultaneous attenuation of the inhibitory effects of clonidine and neuropeptide Y on basal as well as drug-stimulated adenylate cyclase activities of ciliary processes suggest that inhibition of the same G protein function might be a common cause underlying both these effects of pertussis toxin. Moreover, this suggestion is substantiated by the inability of heat-inactivated pertussis toxin to influence any of these two processes.

The question arises, of course, what kind of a G protein is involved in the decrease of IOP lowering effects of clonidine and isoproterenol elicited by pertussis toxin pretreatment. Our results showed a remarkable attenuation of inhibitory effects by pertussis toxin of clonidine and neuropeptide Y on adenylate cyclase activity of ciliary processes. A similar effect of pertussis toxin administered *in vitro* on inhibition of adenylate cyclase of ciliary processes by serotonin has been reported recently (Tobin and Osborne 1989). To date, these two results can be taken as a good indication of the presence of G_i protein in ciliary processes and of its role as a transducer of inhibitory signals from inhibitory α_2-adrenergic and neuropeptide Y receptors to the catalytic unit of adenylate cyclase. Moreover, this suggests that clonidine might bring about its IOP lowering effect by stimulating α_2-adrenergic receptors in ciliary processes which in turn elicits an inhibition of adenylate cyclase and a decrease in the formation of cyclic AMP in ciliary processes. Such an idea would contradict the widely accepted hypothesis of the Sears' group that it is just the stimulation of adenylate cyclase of ciliary processes that can

lead to decreases of IOP (Sears and Mead 1983; Sears et al. 1984). The Sears' hypothesis is grounded on indirect evidence and its weakest point is the fact that it has not offered any plausible explanation for the IOP lowering effect of β-adrenergic blocking drugs. On the contrary, the idea that inhibition of adenylate cyclase of ciliary processes might lead to decrease of IOP is quite consistent with the ocular hypotensive effects of β-blockers. This idea may even offer an explanation for a rather unexpected finding of the attenuation of IOP lowering effect of isoproterenol by pertussis toxin. It seems possible that isoproterenol in 1% concentration might be able to express the α-stimulating component of its action. This in turn could lead to inhibition of adenylate cyclase in ciliary processes and represent that part of isoproterenol IOP lowering effect which is sensitive to the action of pertussis toxin.

We are aware of the fact that the results presented herein may find yet another kind of interpretation since the pertussis toxin is known to inhibit the function of not only G_i protein but also of several other GTP dependent proteins, e.g. G proteins involved in the transduction of signals from postsynaptic receptors to protein kinase C (Dolphin 1987) and to some ion channels (Brown and Birnbaumer 1988). Pertussis toxin is also able to inhibit G protein(s) responsible for signal transduction from presynaptic α_2-adrenergic and other receptors decreasing the release of neurotransmitters (Allgaier et al. 1985). Any of these G proteins can, perhaps, be involved in the regulation of IOP and in the mediation of the effects of drugs influencing IOP.

In summary, the present results show that pertussis toxin administered to rabbits *in vivo* is able to abolish the IOP lowering effect of the α_2-adrenergic agonist clonidine and to attenuate the same effect of the β-adrenergic agonist isoproterenol. This finding strongly suggests the involvement of a pertussis toxin-sensitive G protein in the regulation of IOP and in the effects of several IOP decreasing drugs. *In vivo* administration of pertussis toxin led to a clear-cut decrease of inhibitory effects of clonidine and neuropeptide Y on adenylate cyclase activity of ciliary processes. This finding indicates the presence in ciliary processes of G_i protein which is known to serve as the transducer of inhibitory signals from α_2-adrenergic and neuropeptide Y receptors to adenylate cyclase. Both these findings suggest that the pertussis toxin-sensitive G protein, involved in the mediation of IOP lowering effects of clonidine and isoproterenol, might be the G_i protein. Consequently, inhibition of adenylate cyclase activity in ciliary processes might be responsible for the decrease of IOP. Nevertheless, the participation of other pertussis toxin-sensitive G proteins in IOP lowering effects of either clonidine or isoproterenol or both cannot be ruled out at present.

Acknowledgements. This work was partly supported by the grant Z 207. We are indebted to Dr. C. Wahlestedt from Department of Neurology, The New York Hospital-

Cornell Medical Center, USA, for the generous gift of neuropeptide Y The excellent technical assistance of Miss Lida Kožlova is highly appreciated

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Final version accepted January 21, 1993