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Effects of Drugs Acting on Adrenergic and Adenosine Receptors on the Intraocular Pressure and the Activity of Adenylyl Cyclase in Ciliary Processes and Their Sensitivity to Pertussis Toxin

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Summary

The effects of the selective alpha$_2$-adrenergic agonist p-aminoclonidine, the nonselective adrenergic agonist epinephrine, the selective beta$_2$-adrenergic agonist fenoterol and the adenosine A_1 agonist R-PIA on intraocular pressure were studied in control and pertussis toxin-pretreated rabbits. Pretreatment of rabbits with pertussis toxin decreased the ocular hypotensive effects of p-aminoclonidine and epinephrine, did not influence the same effects of fenoterol or R-PIA and markedly potentiated the initial ocular hypertensive effects of epinephrine and R-PIA. As far as the action on adenylyl cyclase in ciliary processes is concerned, isoproterenol stimulated its activity in control rabbits and epinephrine exerted dual, i.e. stimulatory and inhibitory effects on the activity of this enzyme. The data obtained with epinephrine and p-aminoclonidine confirm the view that their ocular hypotensive effects are associated with their inhibitory action on adenylyl cyclase and contradict the opinion that the hypotensive action of adrenergic drugs depends on adenylyl cyclase activation.

Key words
Adenosine agonists – Adenylyl cyclase – Adrenergic agonists – Intraocular pressure – Pertussis toxin

Introduction

It has been shown that pretreatment of rabbits with pertussis toxin considerably reduces the ocular hypotensive effect of a selective alpha$_2$-adrenergic agonist clonidine (Hynie and Čepelík 1990, 1993). This finding indicates that inhibition of the activity of adenylyl cyclase of ciliary processes might play a role in decreasing intraocular pressure (IOP) elicited by various drugs and, moreover, that the pertussis toxin might be a useful tool in studies of IOP regulation. We decided to corroborate the original finding by studying the influence of the pertussis toxin on the effects of other drugs on IOP.

This paper provides data about the influence of pertussis toxin on the effects of several drugs known to affect different tissues and organs *via* changes in the activity of adenylyl cyclase; we concentrated on the effects of the selective alpha$_2$-adrenergic agonist p-aminoclonidine, the nonselective adrenergic agonist epinephrine and the relatively selective A_1 adenosine agonist R-PIA. The effects of these drugs on adenylyl cyclase activity in ciliary processes were also tested.

Material and Methods

Animals

Pigmented rabbits of the Chinchilla strain (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).

Pertussis toxin pretreatment

A group of rabbits was pretreated by pertussis toxin purified partially by the method of Hynie (1986), in a dose corresponding to 5.0 μg per kg of a commercial preparation of pertussis toxin (List

Biological Laboratories, Inc. Campbell, USA) as estimated according to the lipolytic effect on rat adipose tissue preparations *in vitro* (Hynie 1987). The pertussis toxin was administered into an ear vein. The effects of drugs on intraocular pressure of these animals were estimated in the second and third week after the administration of pertussis toxin.

Intraocular pressure (IOP) measurement

IOP was measured using an applanation pneumatonograph (Digilab Inc., Cambridge, MA) in conscious animals after topical anaesthesia of the cornea with a drop of 0.25 % tetracaine. Drugs tested for the effects on IOP were dissolved in physiological saline and administered topically in a 50 μl volume into the conjunctival sac of one eye. The volume of 50 μl of physiological saline was applied into the contralateral conjunctival sac. The exception was R-PIA, which was dissolved in a 15 % solution of DMSO. In this case 50 μl of 15 % DMSO were applied as the vehicle into the contralateral eye. IOP was measured immediately before application of the appropriate solution and subsequently for several hours at one or two hour intervals. The effects of treatment are expressed as percentage of the control IOP value estimated before treatment with drugs and only the effects in the treated eyes are presented in Figures.

Adenylyl cyclase assay

Homogenates of ciliary processes were prepared as described previously (Čepelík and Hynie 1990). The protein content in homogenates was estimated with Folin-phenol reagent (Lowry *et al.* 1951). Adenylyl cyclase assay was performed using ^{32}P-alpha-ATP as the substrate. Radioactive cyclic AMP formed was separated using aluminum column chromatography as described by Čepelík and Hynie (1990).

Statistical analysis

Data are presented as mean values ± S.E.M. Statistical significance was tested by using Student's t-test. The accepted level of significance for all results was $P<0.05$.

Drugs

(−)-Isoproterenol hydrochloride, R-PIA (R(−)- N^6- (2-phenylisopropyl) adenosine), p-aminoclonidine hydrochloride, (−)-epinephrine d-bitartrate, and fenoterol hydrobromide were from Sigma (St. Louis, USA). Yohimbine hydrochloride was from SPOFA (Prague, Czech Republic).

Commercial preparation of pertussis toxin was product of List Biological Laboratories, Inc. (Campbell, USA). ^{32}P-ATP was prepared in our laboratory by the procedure of Symons (1977). All other chemicals were commercial preparations and were used without further purification.

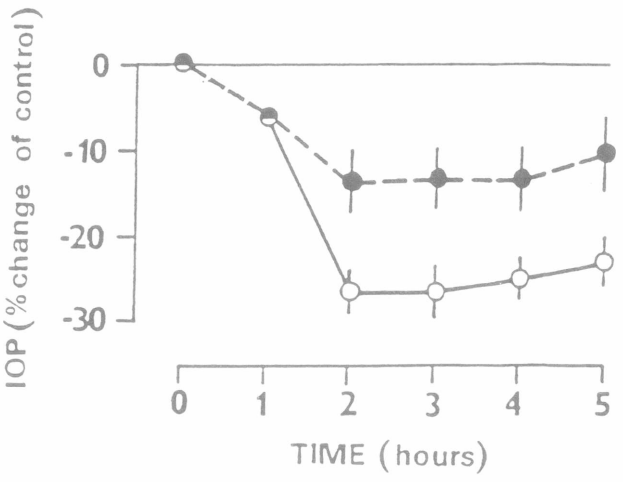

Fig. 1
Comparison of the ocular hypotensive effects of p-aminoclonidine (0.5 %) in the treated eye in control (full line) and pertussis toxin-pretreated rabbits (broken line). Baseline intraocular pressure at time zero was taken as 100 %. Values at 2–6 h in pertussis toxin-pretreated rabbits are significantly different from values of control animals. N = 6 animals.

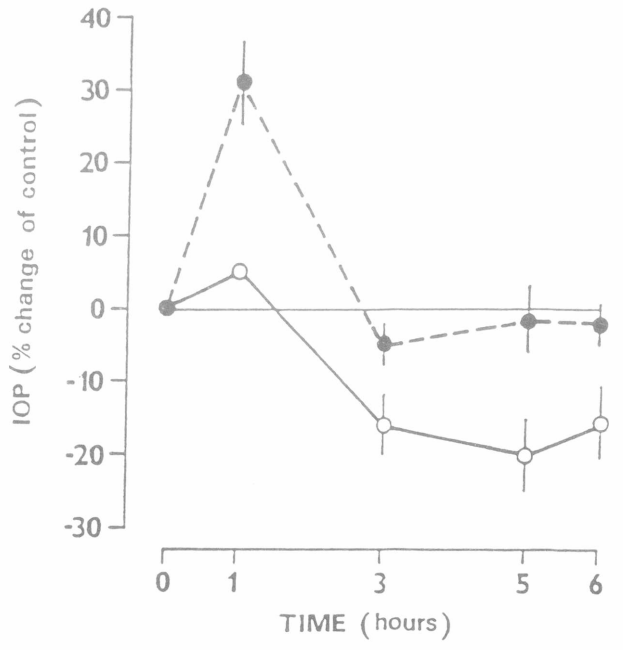

Fig. 2. *The influence of epinephrine (1 %) on the intraocular pressure in control (full line) and pertussis toxin-pretreated rabbits (broken line). Baseline intraocular pressure at time zero was taken as 100 %. Every value in pertussis toxin-pretreated rabbits is significantly different from the corresponding value in control rabbits. N = 6 animals.*

Results

Figure 1 shows that topical application of a selective alpha$_2$-adrenergic agonist p-aminoclonidine significantly decreased intraocular pressure (IOP) in control rabbits. This ocular hypotensive effect of p-aminoclonidine was considerably reduced in pertussis toxin-pretreated rabbits.

A nonselective adrenergic agonist epinephrine induced a small increase of IOP in control rabbits during the first hour after its application which was followed by a considerable decrease of IOP subsequently (Fig. 2). The initial ocular hypertensive effect of epinephrine was markedly enhanced (about six times) in pertussis toxin-pretreated animals but its subsequent ocular hypotensive effect was considerably reduced.

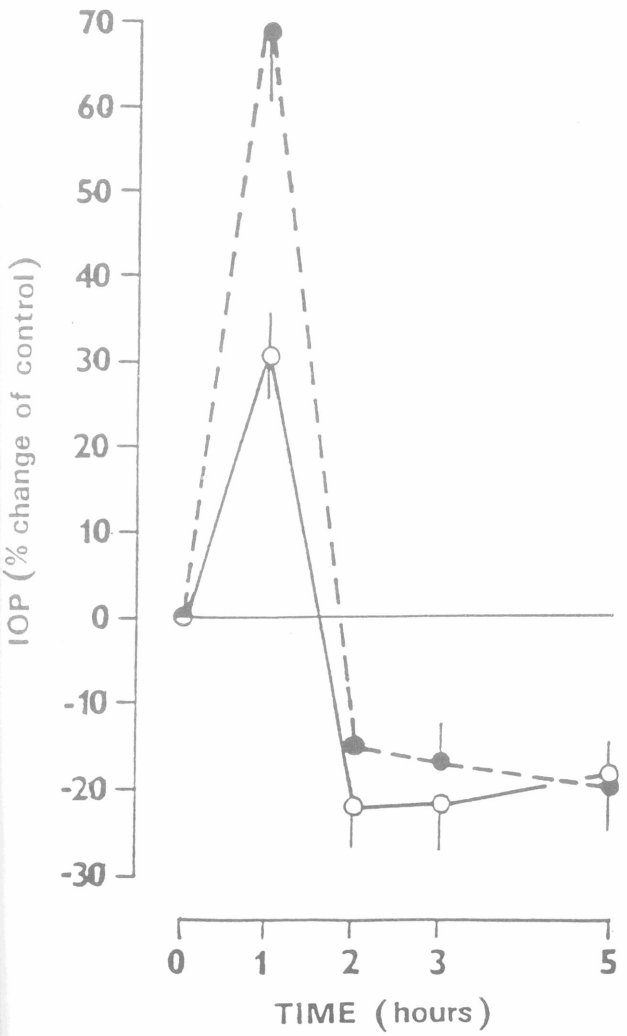

Fig. 3. *The influence of R-PIA (1 %) on the intraocular pressure in control (full line) and pertussis toxin-pretreated rabbits (broken line). Baseline intraocular pressure at time zero was taken as 100 %. Value at 1 h in pertussis toxin-pretreated rabbits is significantly different from the corresponding value in control rabbits. N = 6 animals.*

As is apparent from Figure 3, a relatively selective A_1 adenosine agonist R-PIA elicited a considerable increase of IOP in the first hour after its application, which was subsequently followed by a decrease. The initial ocular hypertensive effect of R-PIA was again remarkably intensified in pertussis toxin-pretreated animals but its eventual ocular hypotensive effect was not influenced.

A selective beta$_2$-adrenergic agonist fenoterol only decreased IOP in both control and pertussis toxin-pretreated rabbits (data not shown).

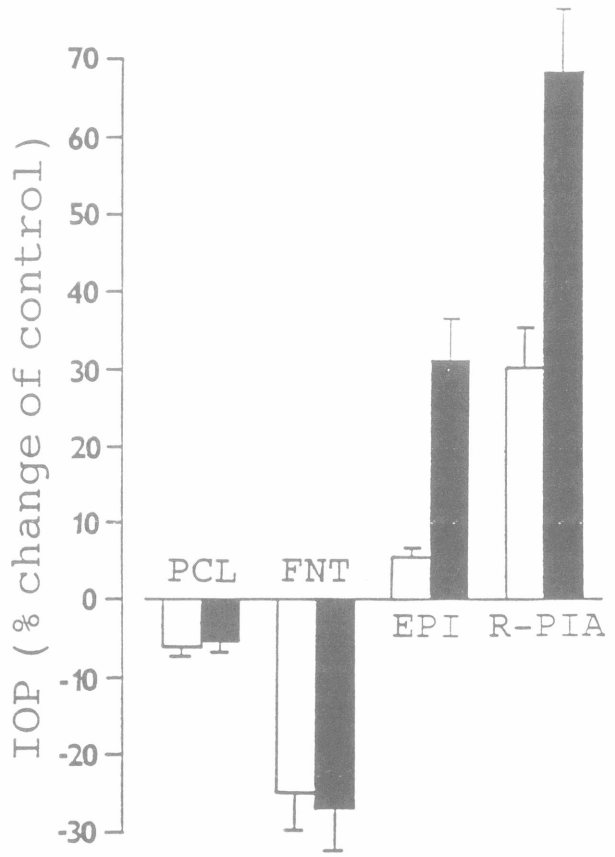

Fig. 4. *Comparison of the effects of p-aminoclonidine (PCL), fenoterol (FNT), epinephrine (EPI) and R-PIA on intraocular pressure at the end of the first hour after their administration in control (empty bars) and pertussis toxin-pretreated rabbits (filled bars). Values of epinephrine and R-PIA in pertussis toxin-pretreated rabbits are significantly different from the corresponding value in control rabbits. N = 6 animals.*

Figure 4 summarizes the influence of pertussis toxin-pretreatment on the effects of drugs on IOP one hour after their administration. Pertussis toxin-pretreatment did not influence the ocular hypotensive effect either of selective alpha$_2$-adrenergic agonist p-aminoclonidine or of selective beta$_2$-adrenergic

agonist fenoterol. However, pertussis toxin-pretreatment considerably intensified the initial ocular hypertensive effect of a relatively selective A_1 agonist R-PIA and especially that of the nonselective adrenergic agonist epinephrine.

The effects of two nonselective adrenergic agonists, i.e. isoproterenol and epinephrine, on the activity of adenylyl cyclase of rabbit ciliary processes both in the presence and absence of a selective alpha$_2$-adrenergic antagonist yohimbine are compared in Figure 5. In the absence of yohimbine, both isoproterenol and epinephrine stimulated the activity of adenylyl cyclase. However, the maximum effect of epinephrine reached only about 50 % of the maximum effect of isoproterenol. In the presence of yohimbine, the effect of isoproterenol was not changed, but the maximal effect of epinephrine was the same as that of isoproterenol.

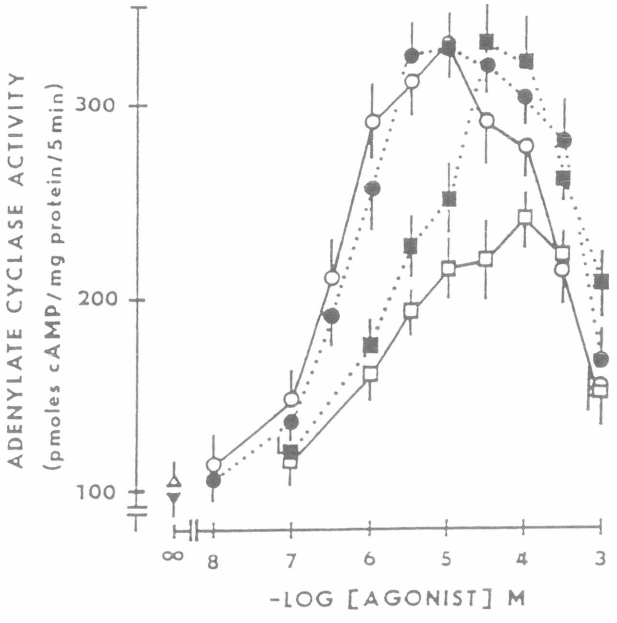

Fig. 5. *The influence of epinephrine (squares) and isoproterenol (circles) in the presence (filled symbols) and absence (empty symbols) of yohimbine (10 μM) on the activity of adenylyl cyclase from ciliary processes of the rabbit eye. N = 6 experiments.*

Furthermore, we found that fenoterol behaved as a full beta$_2$-adrenergic agonist on the preparation of adenylyl cyclase of rabbit ciliary processes (unpublished results), while selective alpha$_2$-adrenergic agonists p-aminoclonidine and clonidine inhibited this enzyme approximately equipotently and R-PIA inhibited its activity only marginally (data not shown).

Discussion

There are two contradictory views concerning the role of adenylyl cyclase in ciliary processes regulating intraocular pressure (IOP). According to the first view, originally proposed by Sears' group, the stimulation of this enzyme leads to a decrease of aqueous humour formation and subsequently to a decrease of IOP (Sears and Mead 1983, Sears *et al.* 1984). According to the second view that is supported by several pieces of indirect evidence inhibition of this enzyme could decrease IOP (Wax 1992, Hynie and Čepelík 1993, WAX *et al.* 1993).

The present results have shown that the selective alpha$_2$-adrenergic agonist p-aminoclonidine and the selective beta$_2$-adrenergic agonist fenoterol decreased intraocular pressure in control rabbits after their topical administration. However, the effects of the nonselective adrenergic agonist epinephrine and the A_1 adenosine agonist R-PIA on IOP were biphasic, i.e. their ocular hypotensive effects were preceded by a clear-cut ocular hypertensive effect expressed in the first hour after their administration. Pretreatment of animals with the pertussis toxin led to a considerable attenuation of the ocular hypotensive effect of p-aminoclonidine and epinephrine, but the effects of fenoterol and R-PIA were the same. However, the most important finding seems to be the marked intensification of the initial ocular hypertensive effect of R-PIA and especially that of epinephrine after pertussis toxin-pretreatment.

The ability of the pertussis toxin to decrease the ocular hypotensive effect of a selective alpha$_2$-adrenergic agonist p-aminoclonidine corresponds to our previously published finding that the pertussis toxin inhibits the ocular hypotensive effect of another selective adrenergic agonist, clonidine (Hynie and Čepelík 1993). This influence of the pertussis toxin both on the ocular hypotensive effect of clonidine and p-aminoclonidine indicates the participation of a pertussis toxin-sensitive G-protein, that serves as a transducer between the inhibitory alpha$_2$-adrenergic receptor and adenylyl cyclase, in ocular hypotensive effects of both these drugs. The inability of the pertussis toxin to inhibit the small ocular hypotensive effect of p-aminoclonidine in the first hour might suggest that the pertussis toxin-sensitive G-protein did not participate in this effect of p-aminoclonidine. The inhibitory influence of the pertussis toxin on the ocular hypotensive effect of epinephrine indicates that this effect of epinephrine may be due to its stimulation of the inhibitory alpha$_2$-adrenergic receptor.

R-PIA is a relatively selective agonist of the adenosine A_1 receptor and has recently been shown to exert an initial ocular hypertensive and subsequent hypotensive response after topical application in control rabbits (Crosson 1992). It has also been shown to exert an inhibitory effect on the production of cyclic

AMP in rabbit iris/ciliary body (Crosson 1995). The lack of the inhibitory effect of the pertussis toxin on its ocular hypotensive effect might possibly indicate that yet another, a pertussis toxin-insensitive mechanism, could participate in the ocular hypotensive effect of R-PIA. Moreover, as mentioned in the section "Results", we were able to prove only marginal inhibition of adenylyl cyclase activity by this drug in rabbit ciliary processes.

The potentiation of the initial ocular hypertensive effect of epinephrine by the pertussis toxin suggests that the ocular hypotensive effect of this drug may be mediated by stimulation of alpha$_2$-adrenergic receptors and subsequent inhibition of adenylyl cyclase activity, possibly in ciliary processes, and that stimulation of beta-adrenergic receptors and the subsequent increase adenylyl cyclase activity, possibly in ciliary processes, may elicit its ocular hypertensive effect.

Pretreatment of animals by the pertussis toxin could block the epinephrine effect on inhibitory alpha$_2$-adrenergic receptors and permit a higher expression of the effect of stimulation of beta-adrenergic receptors leading to potentiation of the initial ocular hypertensive effect of epinephrine. The dual, i.e. stimulatory and inhibitory effect of epinephrine on adenylyl cyclase of ciliary processes has been shown recently (Bausher *et al.* 1987) and confirmed in the present report.

The potentiation of the initial hypertensive action of R-PIA by pertussis toxin treatment might be explained in a similar way as the potentiation of the initial hypertensive action of epinephrine, assuming that a pertussis toxin-sensitive G-protein is involved in the mechanism of the hypotensive action of R-PIA. The ocular hypotensive effect of fenoterol might possibly be due to increased aqueous humour outflow.

In any case, data obtained with epinephrine and p-aminoclonidine are in agreement with the view that their ocular hypotensive effects are associated with their inhibitory action on adenylyl cyclase (Wax 1992, Hynie and Čepelík 1993, Wax *et al.* 1993) and contradict the opinion that the hypotensive action of adrenergic drugs depends on adenylyl cyclase activation (Sears and Mead 1983, Sears *et al.* 1984).

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