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Localisation of prostaglandin $F_{2\alpha}$ and E_2 binding sites in the human eye

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Abstract

Prostaglandin $F_{2\alpha}$ reduces intraocular pressure possibly by increasing uveoscleral outflow. To further understand the mechanism of its action binding sites for prostaglandin $F_{2\alpha}$ and, for comparison, prostaglandin E_2 were localised in sections of human cadaveric eyes using an in vitro ligand-binding technique and autoradiography. Specific binding sites for both prostaglandin $F_{2\alpha}$ and E_2 were co-localised at a high level in the areas of the ciliary muscles and iris sphincter muscles, and at a lower level in the iris epithelium and the retina. The results suggest that prostaglandin $F_{2\alpha}$ and also prostaglandin E_2, could modulate uveoscleral outflow by binding to their receptors located on the ciliary muscles and inducing their relaxation.

Prostaglandin $F_{2\alpha}$ and especially its more lipophilic prodrug prostaglandin $F_{2\alpha}$-1-isopropylester can reduce intraocular pressure (IOP) when applied topically to normal human volunteers[1 2 3] and patients with glaucoma.[4 5] Prostaglandin $F_{2\alpha}$ isopropylester is a very promising drug for glaucoma treatment because it has a prolonged potent hypotensive effect in low doses and lacks serious subjective or objective side effects.

A working classification of prostanoid receptors for five naturally occurring prostaglandins has been proposed based on pharmacological studies in which the agonist potencies of naturally occurring prostaglandins as well as other synthetic agonists and antagonists were compared.[6 7] Receptors sensitive to thromboxane A_2, prostaglandin D_2, E_2, $F_{2\alpha}$, and I_2 have been designated as TP, DP, EP, FP, and IP-receptors, respectively. The EP receptor can be further classified into three subtypes, called EP_1, EP_2, and EP_3 receptors.[8 9] The EP_3 receptor has been shown to be involved in reduction of IOP in rabbits.[10 11] However it is not known which class of prostanoid receptors is involved in the pressure reduction for humans and where within human eyes the receptors for prostaglandin $F_{2\alpha}$ are located.

There is evidence that prostaglandin $F_{2\alpha}$ lowers IOP by increasing uveoscleral outflow in monkeys.[12-14] In order to further understand the basic mechanism of its action it is crucial to reveal the localisation of binding sites (receptors) for prostaglandin $F_{2\alpha}$ in the eye. It is also important to use human eyes since there are considerable anatomical and physiological differences between the eyes of different species, especially in the systems controlling aqueous humour outflow.[15] In this study we localised binding sites for prostaglandin $F_{2\alpha}$ and also for prostaglandin E_2 in human eye sections using an in vitro ligand binding technique and autoradiography.

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Materials and methods

PREPARATION OF HUMAN EYE SECTIONS

Human cadaveric eyes were obtained within 24 hours after death from the Eye Bank of British Columbia. The eyes used in this study had no documented ocular diseases. They were frozen in isopentane cooled to $-80°C$ and stored at $-20°C$ until use. Sections of 20 μm thickness were cut with a cryostat (Cambridge Instruments, Nussloch, Germany) and placed on glass slides coated with 1·7% gelatin.

IN VITRO LIGAND BINDING AND AUTORADIOGRAPHY

The sections were thawed at room temperature and preincubated in 50 mM tris-hydrochloride buffer (pH 7·4) containing 100 mM sodium chloride, 3 mM calcium chloride, and 5% (weight/volume) bovine serum albumin (BSA, Sigma, St Louis, MO, USA) for 60 minutes at room temperature. The sections were then incubated in the same buffer containing 10–20 nM of tritiated prostaglandin $F_{2\alpha}$ or E_2 (Du Pont Canada, Mississauga, Ontario) for 90 minutes at room temperature, washed for 60 minutes in the ice-cold buffer containing 1% BSA, and finally dried in an air stream. The sections were then apposed to tritium-sensitive films (Hyperfilm-^{3}H: Amersham Canada, Oakville, Ontario) for 8 weeks in the dark. Non-specific binding was determined by incubating sections in the buffer containing, in addition, 100 μM unlabelled prostaglandin $F_{2\alpha}$ or prostaglandin E_2 (Cayman Chemicals, Ann Arbor, MI, USA) as well as 10–20 nM of a labelled ligand.

Results

Specific binding sites for both prostaglandin $F_{2\alpha}$ and E_2 were co-localised in the areas of the ciliary muscles and iris sphincter muscles at a high level, and also in the iris epithelium and the retina at a lower level as shown in Figures 1 and 2. The lens structure showed apparently moderate levels of the binding for both prostaglandins, which were not distinctive compared with levels of the corresponding non-specific binding. The results were reproducible in three eyes from three different individuals: a 60-year-old man who died of cerebral infarction, a 70-year-old woman who died of myocardial infarction, and a 52-year-old woman who died of colon cancer.

The binding of 20 nM tritiated prostaglandin $F_{2\alpha}$ in all of the areas could be totally displaced by

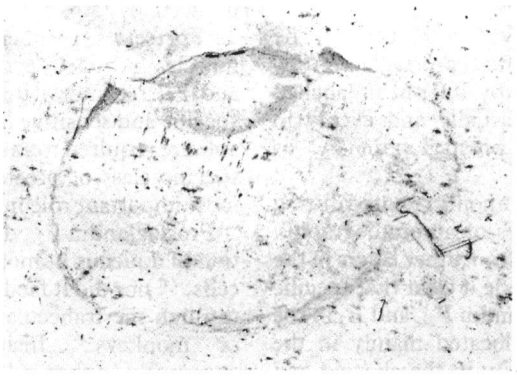

Fig 1A

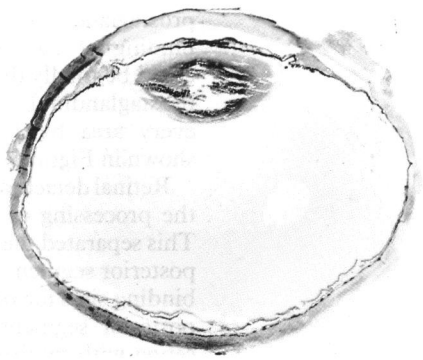

Fig 1C

Fig 1B

Fig 1D

Figure 1 Autoradiographic views of prostaglandin $F_{2\alpha}$ binding sites in eye sections from a 60-year-old man. The binding of 20 nM tritiated prostaglandin $F_{2\alpha}$ (A) concentrates in the ciliary muscle, iris sphincter muscle, iris epithelium, and the retina (and the detached retina). The binding was completely displaced by 100 μM unlabelled prostaglandin E_2 (B) and by 100 μM unlabelled prostaglandin $F_{2\alpha}$ (D). A Nissl-stained view of A is given in C.

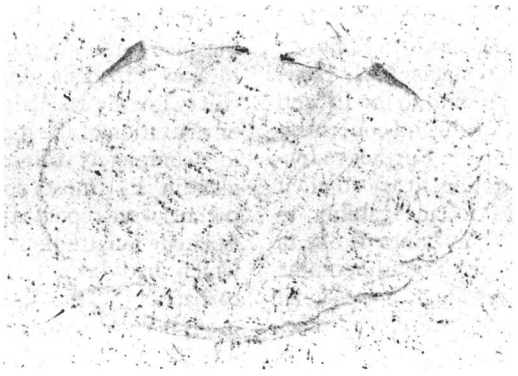

Fig 2A

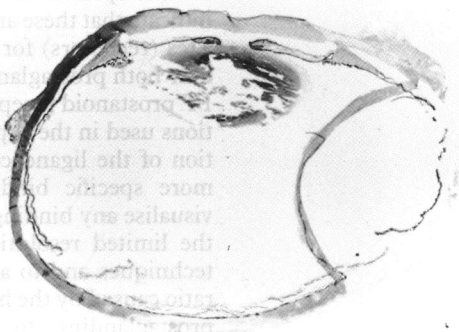

Fig 2C

Fig 2B

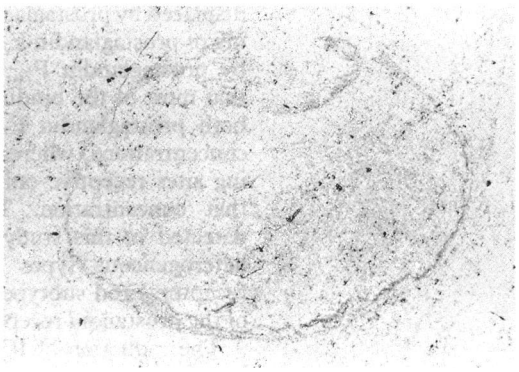

Fig 2D

Figure 2 Autoradiographic views of prostaglandin E_2 binding sites in eye sections from a 60-year-old man. The binding of 20 nM tritiated prostaglandin E_2 (A) concentrates in the ciliary muscle, iris sphincter muscle, iris epithelium, and the retina (and the detached retina). The binding was completely displaced by 100 μM unlabelled prostaglandin E_2 (B), and partially displaced by 100 μM unlabelled prostagladin $F_{2\alpha}$ (D). A Nissl stained view of A is given in C.

100 μM unlabelled prostaglandin $F_{2\alpha}$ and prostaglandin E_2 as shown in Figure 1. The binding of 20 nM tritiated prostaglandin E_2 could be totally displaced by 100 μM unlabelled prostaglandin E_2, and partially and evenly in every area by 100 μM prostaglandin $F_{2\alpha}$ as shown in Figure 2.

Retinal detachment commonly resulted during the processing of the eyes of autoradiography. This separated the retina from other layers in the posterior segment and made it clear that specific binding sites for prostaglandin $F_{2\alpha}$ and E_2 in the posterior segment were located mainly in the retina with minimal density in the choroid and retinal pigment epithelial cells. The resolution of the autoradiographic films was insufficient to determine which specific layer in the retina, if any, showed a high concentration of prostaglandin binding sites.

Discussion

Prostaglandins are known to bind with pigments in the eye tissues.[16] In the present experiments the binding of prostaglandins to pigments could be reduced to less than 10% of the total binding by preincubating and incubating the sections with high concentrations (5%) of BSA and washing the sections with 1% BSA for durations as long as 60 minutes. The conditions used are based on the fact that prostaglandins are lipophilic and bind to the pigments with a weaker affinity than they do to their receptors.

In the present experiments, binding sites for prostaglandin $F_{2\alpha}$ and prostaglandin E_2 were colocalised to the same areas of the eye, primarily the ciliary muscles and iris sphincter muscles, the iris epithelium, and the retina. The results indicate that these areas had a mixture of binding sites (receptors) for both prostaglandins. However both prostaglandins can bind to the EP and FP prostanoid receptors at the ligand concentrations used in the experiments.[6-11] Further reduction of the ligand concentration to try to elicit more specific binding led to an inability to visualise any binding sites. This failure is due to the limited resolution of the autoradiographic techniques and to a relatively high noise/signal ratio caused by the high 'background' binding of prostaglandins to the ocular pigments as mentioned above.

The binding of prostaglandin $F_{2\alpha}$ was totally displaced by prostaglandin E_2, whereas the binding of prostaglandin E_2 was not totally displaced by prostaglandin $F_{2\alpha}$. These results indicated that most of the binding sites were receptive to both prostaglandin $F_{2\alpha}$ and E_2 at the 20 nM concentrations, whereas there were some binding sites receptive only to prostaglandin E_2 at that concentration. Thus the binding sites detected in this study would be a mixture of heterogeneous types (EP receptors and FP receptors) and subtypes (those of EP receptors) of the prostanoid receptors.

The reduction of IOP by prostaglandins has been previously demonstrated to be mediated by stimulation of the EP_3 prostanoid receptor but not of the FP receptor in rabbits.[10 11] It is still unknown which specific class of prostanoid receptors is involved in the pressure reduction induced by prostaglandin $F_{2\alpha}$ in human eyes. The present experiments suggest that the ciliary muscles in human eyes have both EP receptors and FP receptors. Further studies using more specific and sensitive ligands not presently available are required to answer the question of which specific class of prostanoid receptors plays the most important role in ocular hypotension.

Prostaglandin $F_{2\alpha}$ did not change the production of aqueous humour by the ciliary epithelial cells,[17 18] nor did it facilitate conventional outflow through the trabecular meshwork in humans[4 17] or monkeys.[19] Instead prostaglandin $F_{2\alpha}$ apparently enhanced uveoscleral outflow in monkeys[12 13] and its pressure-lowering effect was antagonised by pilocarpine.[14] However the exact mechanism by which it modulates the uveoscleral outflow remains unknown. Uveoscleral outflow can be affected by the tone of the ciliary muscles: contraction of the muscles induced by pilocarpine reduces the uveoscleral flow whereas their relaxation by atropine enhances the flow.[20 21] The binding sites for prostaglandin $F_{2\alpha}$ appeared to be localised along bundles of the ciliary muscle in the ciliary body. It is therefore most probable that receptors for prostaglandin $F_{2\alpha}$ are located on the ciliary muscles.

Repeated doses of prostaglandin $F_{2\alpha}$ produced narrowing of the ciliary muscle fibres and widening of the intermuscular spaces in cynomolgus monkeys[22] and might also cause lysis and loss of intermuscular connective tissue.[23] Its administration was accompanied by partial reversal of resting myopia in monkeys[24] but it did not induce any measurable refractive changes in humans.[3 4]

Although the facts mentioned above suggest that prostaglandin $F_{2\alpha}$ could induce relaxation of the ciliary muscles there has been no evidence as to how prostaglandins would act directly on ciliary muscles, whether they induce contraction or relaxation of the muscles, or whether they act in a completely different way. Recently, prostaglandin E_2 (most potent), D_2 and $F_{2\alpha}$ (less potent) were reported to induce relaxation of the isolated longitudinal ciliary muscles of cats which had been contracted beforehand with carbachol.[25] The autoradiographic techniques used here also demonstrated that the ciliary muscle in cats had binding sites for prostaglandin $F_{2\alpha}$ and E_2 as did in humans (data not shown). It will be necessary to examine further the direct effect of prostaglandins on the ciliary muscle, especially in humans, to fully solve these questions.

Prostaglandin E_2 and $F_{2\alpha}$ can be synthesised by cells lining the trabecular meshwork in humans.[26 27] These prostaglandins would remain active for a while until they flow or are transported out of the eye,[28 29] since ocular tissues in humans so far examined apparently do not contain enzymes which degrade prostaglandins metabolically.[30] The present results support the idea that prostaglandins synthesised in the trabecular meshwork would flow posteriorly in the aqueous humour and reach the ciliary body where they may modulate uveoscleral outflow consequent to binding to their receptors located on the ciliary muscle. Of course there is another possibility, namely, that the ciliary muscle itself might

synthesise prostaglandins under certain conditions and thus might, in effect, turn on the uveoscleral outflow pathway.

The fact that the iris sphincter muscles had binding sites for prostaglandin $F_{2\alpha}$ and E_2 suggests that both prostaglandins might induce miosis although miosis caused by their topical administration has not yet been confirmed clinically.[31]

Prostaglandins play a role in the development of cystoid macular oedema especially after cataract extraction.[32] Binding sites for prostaglandin $F_{2\alpha}$ and E_2 found in the retina might support the role of these compounds in cystoid macular oedema. Prostaglandins are known to be actively transported out of the intraocular fluids by an organic acid transport system located in the ciliary epithelium and retinal capillaries.[28 29] Therefore there is a possibility that some of the binding sites detected in the retina by the ligand-binding method would be the sites of this transporter. This result also suggests a possibility that prostaglandin $F_{2\alpha}$ and E_2 could play roles as retinal neuromodulators as has been shown in the brain.[33]

Receptor proteins for prostaglandin E_2 and $F_{2\alpha}$ have been solubilised and shown to be coupled with guanine nucleotide binding proteins (G proteins).[34] Recently a gene for the human thromboxane A_2 receptor has been cloned and shown to belong to the superfamily of G protein-coupled receptor proteins. Expression of this cloned gene for thromboxane A_2 revealed that the receptor responded only to thromboxane A_2 agonists but not to prostaglandin D_2 or $F_{2\alpha}$, supporting the working hypothesis that there might be a separate receptor for each of the naturally occurring prostanoids. In the future molecular biological approaches will further enhance our understanding of the action of prostaglandins in ocular tissues.

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