ET_{B2} RECEPTOR SUBTYPE STIMULATION RELAXES THE IRIS SPHINCTER MUSCLE

by

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Short title: ET_{B2} stimulation relaxes the iris sphincter muscle

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ABSTRACT

Effects of ET_B receptor stimulation and its subcellular pathways were evaluated in carbachol pre-contracted rabbit iris sphincter muscles (n=51). ET_B stimulation with sarafotoxin (SRTX-c; 10^{-10}–10^{-6}M) was tested in the absence (n=7) or presence of 10^{-5}M of: BQ-788 (ET_{B2} receptor antagonist; n=6), L-NA (NOS inhibitor; n=7) or indomethacin (ciclooxygenase inhibitor; n=10). Effects of ET_B stimulation by endothelin-1 (ET-1; 10^{-10}–10^{-7}M) in presence of an ET_A receptor antagonist (BQ-123; 10^{-5}M; n=7) and of ET_{B1} stimulation by IRL-1620 (10^{-10}–10^{-7}M; n=7) were also tested. Finally, the effects of SRTX-c (10^{-9}–10^{-7}M) in electric field stimulation (EFS) contraction were evaluated (n=7). ET_B receptor stimulation by SRTX-c or ET-1 in presence of BQ-123 promoted a concentration-dependent relaxation of the rabbit iris sphincter muscles averaging 10.8±2.0% and 9.4±1.8%,respectively. This effect was blocked by BQ-788 (-2.3±2.0%), L-NA (4.5±2.3%) or indomethacin (2.3±2.9%). Selective ET_{B1} stimulation by IRL-1620 did not relax the iris sphincter muscle (0.9±5.4%). EFS elicited contraction was not altered by SRTX-c. In conclusion, ET_B receptor stimulation relaxes the carbachol pre-contracted iris sphincter muscle, an effect that is mediated by the ET_{B2} receptor subtype, through NO and prostaglandins release.

Keywords: peptide hormones, iris, muscle fibers, ET_{B2}, sarafotoxin-s6c

Abbreviations: SRTX-c: Sarafotoxin s6c; ET –Endothelin; ETA: Endothelin receptor type A; ETB : Endothelin receptor type B; COX – ciclooxygenase; NO: nitric oxide.

INTRODUCTION

Endothelin-1 (ET-1) is an endogenous vasoactive peptide with 21 aminoacids, secreted by vascular endothelial cells (Yanagisawa, M. et al. 1989, Yanagisawa, M. et al. 1988). ET-1 is a member of related peptides family, which includes endothelin-2 (ET-2), endothelin-3 (ET-3), sarafotoxin-s6c (SRTX-c) and vasoactive intestinal contractors. This peptide promotes potently the contraction of both vascular and non-vascular smooth muscles (Eglen, R. M. et al. 1989).

In humans, the 3 isoforms of ET mediate their biological actions by two different receptors, ET_A and ET_B (Arai, H. et al. 1990, Inoue, A. et al. 1989, Sakurai, T. et al. 1990)The ET_A receptor, located on vascular smooth muscle, mediates a potent vasoconstrictor action, promotes miosis and mitogenesis, and binds preferably to ET-1(Masaki, T. 1991), while the ET_B receptor binds equi-potently the three isoforms and mediates vasodilatation and ocular hypotension (Haque, M. S. et al. 1995), probably through stimulation of nitric oxide and prostaglandins release (Inoue, A. et al. 1989, Masaki, T. 1991). This receptor has two isotypes: ET_{B1} or endothelial, and ET_{B2} or muscular (Nishiyama, M. et al. 1995, Sudjarwo, S. A. et al. 1994). Another receptor, the ET-C receptor, cloned from Xenopus melanophores has greater affinity for ET-3 than for ET-1 and ET-2 (Karne, S. et al. 1993, Yorio, T. et al. 2002).

Endothelin-1 is widely distributed in mammalian ocular tissues including cornea, ciliary body epithelium and retina (Ripodas, A. et al. 2001, Yorio, T. et al. 2002). ET-1 mRNA was identified by *in situ* hybridization in human ciliary body, ciliary muscle, constrictor of iris sphincter, stroma and iris vessels. ET-1 was detected in aqueous humour, endothelial and non pigmented ciliary epithelium (Fernandez-Durango, R. et al. 2003). In terms of ET receptors expression in the human iris, almost

2/3 of them are of the ET_B subtype (Fernandez-Durango, R. et al. 2003). The iris is the main expressing tissue of ET receptors, followed by the ciliary muscle and ciliary processes (Fernandez-Durango, R. et al. 2003). Immunoreactivity studies detected ET-3 in the retina (De Juan, J. A. et al. 1995). In the iris of the pig and cat eyes, ET produces a concentration dependent contraction mediated by the ET_A receptor subtype (Geppetti, P. et al. 1989). When injected in the posterior compartment of the eye, ET promotes iNOS stimulation, ischemia of optic nerve and lowering of axonal transport, with destruction of optic nerve and increase of intraocular pressure (IOP) (Yorio, T. et al. 2002). ET administration in the eye's anterior segment reduces IOP, independently of prostaglandin production (Haque, M. S. et al. 1995). Also the production of aqueous humour is affected by ET-1, which inhibits the NA^+/K^+ ATPase (Prasanna, G. et al. 2001).

The aim of this work was to determine the role of ET_B receptor stimulation in the modulation of iris sphincter muscle contraction, its sub-cellular pathways and the ET_B receptor subtype involved.

METHODS

All animal procedures were performed in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

FUNCTIONAL STUDIES

Specimens preparation

The study was performed in isolated iris sphincter (n=51) muscles from male New Zealand white rabbits (*Oryctolagus cuniculus*; 2.0-3.0Kg). Animals were euthanized after an injection of pentobarbital sodium salt (50 mg/kg) into the marginal

ear vein. The eyes were immediately enucleated and placed in modified Krebs-Ringer (KR) solution at 35 ºC, with the following composition in mM: NaCl 98; KCl 4.7; $MgSO_4.7H_2O$ 2.4; KH_2PO_4 1.2; glucose 4.5; $CaCl_2.2H_2O$ 2.5; $NaHCO_3$ 17; $C_3H_3NaO_3$ 15 and CH_3COONa 5. After removal of the cornea, the iris sphincter muscle was quickly excised and immersed in the KR solution. After dissection, the ends of each piece were tied with silk thread for mounting in a 15 ml plexi glass organ bath containing the above-described solutions. One end of the specimen was connected to an electromagnetic length-tension transducer (University of Antwerp, Belgium), and the other was secured to a clip at the wall of the organ bath. All the surgical procedures were taken under microscope (Zeiss, Stemi 2000C, Germany). Solutions were bubbled with 95% O_2 and 5% CO_2 and pH was maintained between 7.38-7.42.

Iris sphincter muscles were stabilized always at the same preload (1.0 mN) and bathing solutions were continuously replaced until muscle length stabilization. They were then switched to isometric conditions and the protocols initiated when muscle tension stabilized.

Experimental protocols

Effect of ET_B stimulation on the pre-contracted iris sphincter muscle

After stabilization, the rabbit iris sphincter muscles were contracted by adding carbachol (10^{-6} M) to the organ bath. The effects of ET_B receptor stimulation on the pre-contracted iris sphincter muscle were studied by evaluating its response to: (i) the ET_B agonist SRTX-c (10^{-10}–10^{-6}M; n=7), (ii) Endothelin-1 (ET-1;10^{-10}–10^{-7} M; n=7) in the presence of an ETA receptor antagonist (BQ-123; 10^{-5} M) (iii) the selective ET_{B1} agonist IRL-1620 (10^{-10}–10^{-7} M; n=7). Furthermore, the response to SRTX-c (10^{-10}–10^{-6}M) was also assessed in the presence of: (i) an ET_{B2} receptor antagonist, BQ-788 (10^{-5} M; n=6); (ii) a NO synthase inhibitor, L-nitro-L-arginine (L-NA; 10^{-5} M; n=7); (iii) a ciclooxygenase inhibitor,

indomethacin (indo; 10^{-5} M; n=10). In each muscle, two carbachol-induced contractions were studied. One of these contractions was randomly selected to test the effects of the drugs to be studied, while the other one was used as control, having been studied in the presence of the vehicle solution alone. In each protocol, each concentration was added to the bath solution only after recording the maximal effect of the previous one.

Effect of ET_B stimulation on the EFS elicited contraction

After stabilization, rabbit iris sphincter muscles (n=7) were contracted by placing them in an electric field stimulation of 10V, 5 Hz and 1 ms duration. Developed tension was recorded in 5 consecutive, 3 minutes apart, contractions. After completing the acquisition in baseline conditions, SRTX-c (10^{-9}) was added to the organ bath and a new electric field stimulation elicited 15 minutes later. The drug was then washed out and a new control contraction obtained. After that, the second concentration of SRTX-c (10^{-8}) was added to the bath and a new electric field stimulation elicited 15 minutes later. The procedure was then repeated to test the concentration of 10^{-7} M. Finally, the drug was washed out again and another control contraction was obtained to confirm the preservation of muscle performance.

Materials

All chemicals were obtained from Sigma Chemical Co (St Louis, Mo). Peptides were prepared in aliquots and stored at -20ºC.

STATISTICAL ANALYSIS

Data presented as means±SEM. EFS-elicited contractions, in presence and absence of SRTX-c, were compared with a paired Student's t-test. Concentration-

response curves of SRTX-c in carbachol pre-contracted muscles in each experimental condition were evaluated with one-way repeated measures ANOVA. Effects of each dose of drug in different experimental conditions were tested with one-way ANOVA. When significant differences were detected with any of the ANOVA test, the Student-Newman-Keuls test was selected to perform multiple comparisons. $P<0.05$ was accepted as significant.

RESULTS

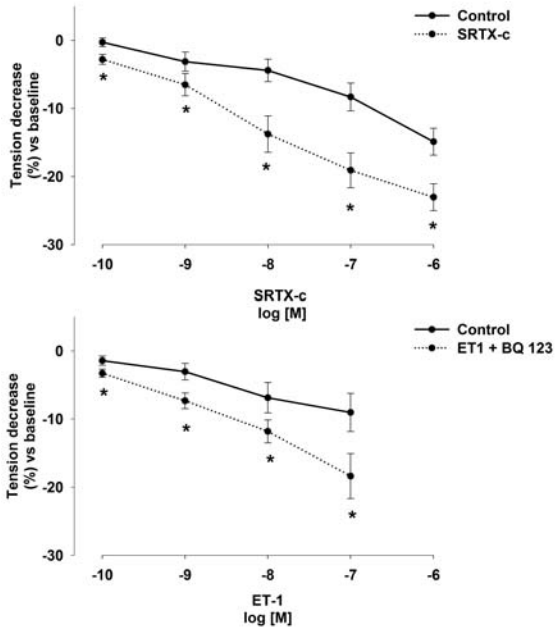
Effect of ET_B stimulation on the carbachol elicited contraction of the iris sphincter muscle

Active tension of the iris sphincter muscle preparations elicited by the addition of carbachol (10^{-6}M) to the bath was quite stable, not significantly different between the different protocols and similar in control and test contractions, averaging 2.99±0.10 mN.

Addition of SRTX-c to the pre-contracted iris sphincter muscle promoted a 19.1±2.6% decrease of the active tension, while the in the control contraction it decreased 8.3±2.0% ($p<0.05$), over the same period of time (figure 1; upper panel).

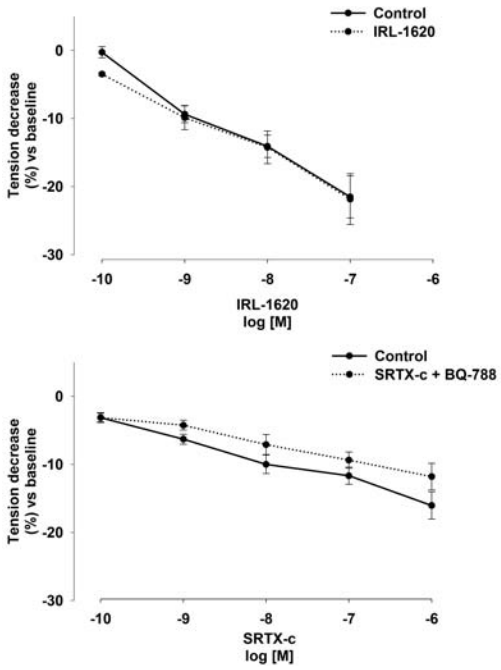
ET_B receptor stimulation by ET-1 in presence of BQ-123 decreased active tension by 16.4±1.8%, while the control contraction decreased only 9.02±2.8 % ($p<0.05$), over the same period of time (figure 1; lower panel).

Figure 1



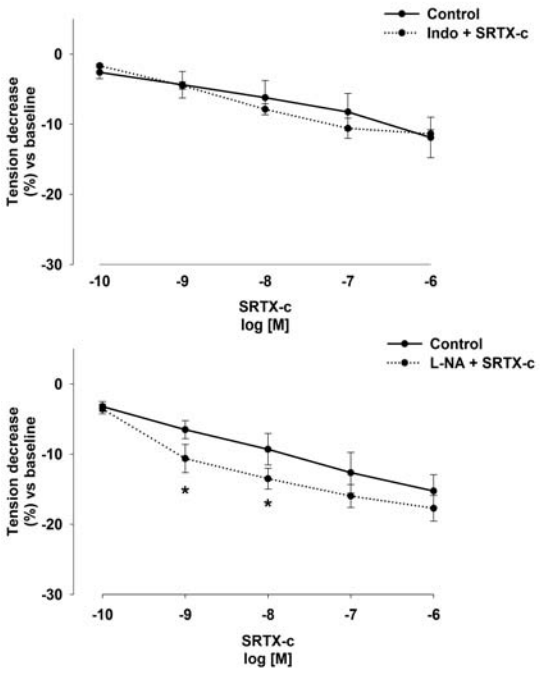
On the contrary, selective ET_{B1} receptor stimulation either by IRL-1620 or by SRTX-c in presence of BQ-788 (figure 2) did not promote a decline in active tension significantly different from the control contraction in the presence of the vehicle alone.

Figure 2



To test the influence of prostaglandins and NO in ET_B induced relaxation of carbachol-induced contraction of the iris sphincter muscle, increasing concentration of SRTX-c were tested in the presence of indomethacin or L-nitro-L-arginine (L-NA). While indomethacin completely inhibited the relaxing effect of SRTX-c (figure 3; upper panel), L-NA only attenuated such effect at concentrations of SRTX-c equal or higher than 10^{-7}M.

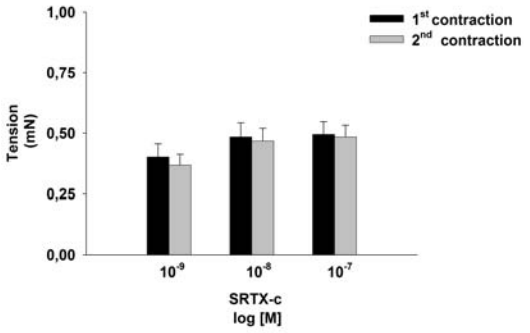
Figure 3



Effect of ET_B stimulation on the EFS elicited contraction

Active tension of the iris sphincter muscle preparations elicited by the EFS was quite stable not significantly different between the different protocols and similar in control and test contractions, averaging 0.45±0.02 mN. The presence of SRTX-c in the bath (10^{-9}–10^{-7}M) did not promote any change in muscle tension, elicited by the electric field stimulation (figure 4).

Figure 4



DISCUSSION

The present study described the relaxation of the pre-contracted iris sphincter muscle promoted by ET_B receptor stimulation. Interestingly, this effect is mediated by the ET_{B2} receptor subtype through prostaglandins and NO release.

In the rat, ET_A receptor stimulation contracts the iris sphincter muscle and potentiates its electric field elicited contraction (Shinkai, M. et al. 1994). In the same experimental preparation, Shinkai-Goromaru and collaborators reported a 140% increase of electric field stimulation developed tension, in response to ET_B receptor stimulation by SRTX-c (Shinkai-Goromaru, M. et al. 1997). In these conditions, ET_B receptor stimulation increased acetylcholine release in the pre-junctional site of the cholinergic synapses. This finding is quite relevant as ET-3, which has a similar affinity for ET_A and ET_B receptors, is more abundant in the iris than ET-1 (Fernandez-Durango, R. et al. 2003, Shinkai-Goromaru, M. et al. 1997). On the contrary, in our work, ET_B receptor stimulation promoted relaxation of the carbachol pre-contracted iris sphincter muscle. This was observed in response to either SRTX-c or ET-1 in presence of BQ-123, an ET_A receptor blocker. This observation suggests that the effects of ET_B

stimulation are distinct in carbachol and EFS elicited contractions. However, in our study the latter was not affected by SRTX-c (10^{-9}–10^{-7}M), suggesting that, contrary to the rat, in the rabbit ET_B receptor stimulation does not increase acetylcholine release.

Table 1- Effects of ET_B stimulation and its subcellular pathway in the iris sphincter muscle

	Δ TENSION (% vs control)	**p<0.05**
SRTX-c	-10.8 ± 2.03 %	vs control
ET1+BQ123	-9.35 ± 1.79 %	vs control
IRL-1620	-0.91 ± 5.45 %	*vs SRTX-c; ET-1*
SRTX-c + BQ-788	2.30 ± 2.04 %	vs SRTX-c; ET-1
SRTX-c + LNA	-4.46 ± 2.28 %	vs SRTX-c
SRTX-c + Indo	-2.55 ± 3.00 %	vs SRTX-c

The presence, in the muscle and endothelium, of two functionally distinct ET_B receptors (ET_{B1} and ET_{B2}) was initially described in the swine pulmonary vein (Sudjarwo, S. A. et al. 1993). Later, additional evidences of the existence of two ET_B receptor subtypes were reported in the rabbit venous saphenous muscle (Nishiyama, M. et al. 1995), in the tracheal smooth muscle (Yoneyama, T. et al. 1995), in the rabbit basilar artery (Zuccarello, M. et al. 1999), in the guinea pig ileum (Miasiro, N. et al. 1999) and in the rabbit heart (Leite-Moreira, A. F. and C. Bras-Silva 2004). The ET_{B2} receptor subtype promotes contraction of the swine pulmonary vein (Sudjarwo, S. A. et al. 1993), the rabbit saphenous vein (Nishiyama, M. et al. 1995), the rabbit basilar artery (Zuccarello, M. et al. 1999) and in the tracheal smooth muscle (Yoneyama, T. et al. 1995). It has a biphasic effect, i.e. relaxation followed by contraction, on the guinea pig ileum (Miasiro, N. et al. 1999, Miasiro, N. et al. 1998), and increases myocardial inotropy in the rabbit heart (Leite-Moreira, A. F. and C. Bras-Silva 2004). SRTX-c acts preferentially in the ET_{B2} receptor subtype, while IRL-1620 selectively stimulates the

ET_{B1} subtype (Karaki, H. et al. 1994a, Karaki, H. et al. 1994b, Sudjarwo, S. A. et al. 1993, Sudjarwo, S. A. et al. 1994, Yoneyama, T. et al. 1995). In our study, we observed that SRTX-c, but not IRL-1620 relaxed the carbachol pre-contracted muscle, an effect that was blocked by BQ-788. These findings suggest that the receptor subtype involved in ET_B induced iris sphincter relaxation is the ET_{B2}. The effect of SRTX-c on ET_{B2}-induced rabbit vein contraction was also previously shown to be inhibited by BQ-788(Karaki, H. et al. 1994a). Interestingly, however, the iris sphincter was the first muscle where a relaxing instead of a contracting effect was described in response to ET_{B2} receptor stimulation.

The relaxing effect of ET_{B2} stimulation was dependent of prostaglandins and NO. These agents also mediate the negative inotropic (Leite-Moreira, A. F. and C. Bras-Silva 2004) and the venous vasodilatory (De Nucci, G. et al. 1988, Filep, J. G. et al. 1991, Hirata, Y. et al. 1993) effects induced by ET_{B1} receptor stimulation. Prostaglandins and their receptors are widely distributed in the ocular tissues, including the iris sphincter muscle. The EP_2 receptor is the most abundant in rabbit (Bhattacherjee, P. et al. 1993, Csukas, S. et al. 1992), mouse and human iris (Biswas, S. et al. 2004). In human eyes, FP, DP and EP receptors were localized in the ciliary body and iris, being particularly involved in the IOP regulation (Davis, T. L. and N. A. Sharif 1999, Sharif, N. A. et al. 2004, Sharif, N. A. et al. 2000).

FP-receptors agonists promoted phosphoinositide (PI) hydrolysis, mitogen-activated protein kinase (MAPK) activation and myiosin light chain phosphorylation causing Ca^{2+} mobilization and iris contraction (Ansari, H. R. et al. 2004, Sharif, N. A. et al. 2008). On the other side, EP_2, EP_4 and DP receptors activation produces intracellular cAMP accumulation and muscle relaxation (Abdel-Latif, A. A. 2001). In our preparation, prostaglandins released by ETB_2 stimulation partially reverse the

carbachol-induced contraction. Further investigations are needed to indentify the subcellular pathway of this effect.

The blockage of endogenous NO production also inhibited SRTX-c induced relaxation. However, in contrast to prostaglandins that mediated relaxation over the entire concentration-response curve, NO dependence was only evident for concentrations of SRTX-c higher than 10^{-7} M. In the bovine iris muscle, a non-adrenergic, non-cholinergic system (Pianka, P. et al. 2000) was shown to promote a NO dependent relaxation. NO release can occur directly from nitrergic neurons (Wiencke, A. K. et al. 1994) or in response to substances such as adrenomedullin that promote its synthesis (Uchikawa, Y. et al. 2005).

This relaxing effect adds to other ET_B receptor mediated effects in ocular tissues, such as the relaxation of the bovine ciliary muscle (Kamikawatoko, S. et al. 1995) and the ocular hypotensive effect (Haque, M. S. et al. 1995). In conjunction with ET-3 levels in the iris and ciliary tissues and with ET-1 levels in exfoliation syndrome patients (Koliakos, G. G. et al. 2004) our results highlight the importance of endothelin (and ET_B pathway) as a regulator of the iris muscles.

FIGURE LEGENDS

Figure 1. Concentration-response curves of SRTX-c (10^{-10}–10^{-6}M; upper panel) and ET-1 (10^{-10}–10^{-7}M) in the presence of BQ-123 (10^{-5}M; lower panel) elicited tension decrease in carbachol pre-contracted iris sphincter muscles. Control lines refer to recordings in presence of the vehicle alone. $p<0.05$: * vs. control.

Figure 2. Concentration-response curves of SRTX-c (10^{-9}–10^{-6}M) in presence of BQ-788 (10^{-5}M; upper panel) and IRL-1620 (10^{-10}–10^{-7}M; lower panel) elicited tension decrease in carbachol pre-contracted iris sphincter muscles. Control lines refer to recordings in presence of the vehicle alone. $p<0.05$: * vs. control.

Figure 3. Concentration-response curves of SRTX-c (10^{-9}–10^{-6}M) in presence of indomethacin (upper panel; 10^{-5}M) or L-nitro-L-Arginine (lower panel; 10^{-5}M) elicited tension decrease in carbachol precontracted iris sphincter muscles. Control lines refer to recordings in presence of the vehicle alone. $p<0.05$: * vs. control.

Figure 4. Graphic representation of tension development in response to two consecutive EFS group of contractions in the absence or presence of SRTX-c (10^{-9}–10^{-7}M).

REFERENCES

ABDEL-LATIF A A: Cross talk between cyclic nucleotides and polyphosphoinositide hydrolysis, protein kinases, and contraction in smooth muscle. *Exp Biol Med (Maywood)* **226**: 153-163, 2001.

ANSARI H R, KADDOUR-DJEBBAR I and ABDEL-LATIF A A: Effects of prostaglandin F2alpha, latanoprost and carbachol on phosphoinositide turnover, MAP kinases, myosin light chain phosphorylation and contraction and functional existence and expression of FP receptors in bovine iris sphincter. *Exp Eye Res* **78**: 285-296, 2004.

ARAI H, HORI S, ARAMORI I, OHKUBO H and NAKANISHI S: Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* **348**: 730-732, 1990.

BHATTACHERJEE P, RHODES L and PATERSON C A: Prostaglandin receptors coupled to adenylyl cyclase in the iris-ciliary body of rabbits, cats and cows. *Exp Eye Res* **56**: 327-333, 1993.

BISWAS S, BHATTACHERJEE P and PATERSON C A: Prostaglandin E2 receptor subtypes, EP1, EP2, EP3 and EP4 in human and mouse ocular tissues--a comparative immunohistochemical study. *Prostaglandins Leukot Essent Fatty Acids* **71**: 277-288, 2004.

CSUKAS S, BHATTACHERJEE P, RHODES L and PATERSON C A: Prostaglandin E2 binding site distribution and subtype classification in the rabbit iris-ciliary body. *Prostaglandins* **44**: 199-208, 1992.

DAVIS T L and SHARIF N A: Quantitative autoradiographic visualization and pharmacology of FP-prostaglandin receptors in human eyes using the novel phosphor-imaging technology. *J Ocul Pharmacol Ther* **15**: 323-336, 1999.
DE JUAN J A, MOYA F J, FERNANDEZ-CRUZ A and FERNANDEZ-DURANGO R: Identification of endothelin receptor subtypes in rat retina using subtype-selective ligands. *Brain Res* **690**: 25-33, 1995.
DE NUCCI G, THOMAS R, D'ORLEANS-JUSTE P, ANTUNES E, WALDER C, WARNER T D and VANE J R: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci U S A* **85**: 9797-9800, 1988.
EGLEN R M, MICHEL A D, SHARIF N A, SWANK S R and WHITING R L: The pharmacological properties of the peptide, endothelin. *Br J Pharmacol* **97**: 1297-1307, 1989.
FERNANDEZ-DURANGO R, ROLLIN R, MEDIERO A, ROLDAN-PALLARES M, GARCIA FEIJO J, GARCIA SANCHEZ J, FERNANDEZ-CRUZ A and RIPODAS A: Localization of endothelin-1 mRNA expression and immunoreactivity in the anterior segment of human eye: expression of ETA and ETB receptors. *Mol Vis* **9**: 103-109, 2003.
FILEP J G, BATTISTINI B, COTE Y P, BEAUDOIN A R and SIROIS P: Endothelin-1 induces prostacyclin release from bovine aortic endothelial cells. *Biochem Biophys Res Commun* **177**: 171-176, 1991.
GEPPETTI P, PATACCHINI R, MEINI S and MANZINI S: Contractile effect of endothelin on isolated iris sphincter muscle of the pig. *Eur J Pharmacol* **168**: 119-121, 1989.
HAQUE M S, TANIGUCHI T, SUGIYAMA K, OKADA K and KITAZAWA Y: The ocular hypotensive effect of the ETB receptor selective agonist, sarafotoxin S6c, in rabbits. *Invest Ophthalmol Vis Sci* **36**: 804-808, 1995.
HIRATA Y, EMORI T, EGUCHI S, KANNO K, IMAI T, OHTA K and MARUMO F: Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. *J Clin Invest* **91**: 1367-1373, 1993.
INOUE A, YANAGISAWA M, KIMURA S, KASUYA Y, MIYAUCHI T, GOTO K and MASAKI T: The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci U S A* **86**: 2863-2867, 1989.
KAMIKAWATOKO S, TOKORO T, AZUMA H, HAMASAKI H and ISHIDA A: The effects of endothelin-1 on isolated bovine ciliary muscles. *Exp Eye Res* **61**: 559-564, 1995.
KARAKI H, SUDJARWO S A and HORI M: Novel antagonist of endothelin ETB1 and ETB2 receptors, BQ-788: effects on blood vessel and small intestine. *Biochem Biophys Res Commun* **205**: 168-173, 1994a.
KARAKI H, SUDJARWO S A, HORI M, TANAKA T and MATSUDA Y: Endothelin ETB receptor antagonist, RES-701-1: effects on isolated blood vessels and small intestine. *Eur J Pharmacol* **262**: 255-259, 1994b.
KARNE S, JAYAWICKREME C K and LERNER M R: Cloning and characterization of an endothelin-3 specific receptor (ETC receptor) from Xenopus laevis dermal melanophores. *J Biol Chem* **268**: 19126-19133, 1993.
KOLIAKOS G G, KONSTAS A G, SCHLOTZER-SCHREHARDT U, HOLLO G, MITOVA D, KOVATCHEV D, MALOUTAS S and GEORGIADIS N: Endothelin-1 concentration is increased in the aqueous humour of patients with exfoliation syndrome. *Br J Ophthalmol* **88**: 523-527, 2004.
LEITE-MOREIRA A F and BRAS-SILVA C: Inotropic effects of ETB receptor stimulation and their modulation by endocardial endothelium, NO, and prostaglandins. *Am J Physiol Heart Circ Physiol* **287**: H1194-1199, 2004.
MASAKI T: Tissue specificity of the endothelin-induced responses. *J Cardiovasc Pharmacol* **17 Suppl 7**: S1-4, 1991.

MIASIRO N, KARAKI H, MATSUDA Y, PAIVA A C and RAE G A: Effects of endothelin ET(B) receptor agonists and antagonists on the biphasic response in the ileum. *Eur J Pharmacol* **369**: 205-213, 1999.
MIASIRO N, KARAKI H and PAIVA A C: Distinct endothelin-B receptors mediate the effects of sarafotoxin S6c and IRL1620 in the ileum. *J Cardiovasc Pharmacol* **31 Suppl 1**: S175-178, 1998.
NISHIYAMA M, MOROI K, SHAN L H, YAMAMOTO M, TAKASAKI C, MASAKI T and KIMURA S: Two different endothelin B receptor subtypes mediate contraction of the rabbit saphenous vein. *Jpn J Pharmacol* **68**: 235-243, 1995.
PIANKA P, ORON Y, LAZAR M and GEYER O: Nonadrenergic, noncholinergic relaxation of bovine iris sphincter: role of endogenous nitric oxide. *Invest Ophthalmol Vis Sci* **41**: 880-886, 2000.
PRASANNA G, DIBAS A, HULET C and YORIO T: Inhibition of Na(+)/K(+)-atpase by endothelin-1 in human nonpigmented ciliary epithelial cells. *J Pharmacol Exp Ther* **296**: 966-971, 2001.
RIPODAS A, DE JUAN J A, ROLDAN-PALLARES M, BERNAL R, MOYA J, CHAO M, LOPEZ A, FERNANDEZ-CRUZ A and FERNANDEZ-DURANGO R: Localisation of endothelin-1 mRNA expression and immunoreactivity in the retina and optic nerve from human and porcine eye. Evidence for endothelin-1 expression in astrocytes. *Brain Res* **912**: 137-143, 2001.
SAKURAI T, YANAGISAWA M, TAKUWA Y, MIYAZAKI H, KIMURA S, GOTO K and MASAKI T: Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* **348**: 732-735, 1990.
SHARIF N A, KADDOUR-DJEBBAR I and ABDEL-LATIF A A: Cat iris sphincter smooth-muscle contraction: comparison of FP-class prostaglandin analog agonist activities. *J Ocul Pharmacol Ther* **24**: 152-163, 2008.
SHARIF N A, WILLIAMS G W, CRIDER J Y, XU S X and DAVIS T L: Molecular pharmacology of the DP/EP2 class prostaglandin AL-6598 and quantitative autoradiographic visualization of DP and EP2 receptor sites in human eyes. *J Ocul Pharmacol Ther* **20**: 489-508, 2004.
SHARIF N A, WILLIAMS G W and DAVIS T L: Pharmacology and autoradiography of human DP prostanoid receptors using [(3)H]-BWA868C, a DP receptor-selective antagonist radioligand. *Br J Pharmacol* **131**: 1025-1038, 2000.
SHINKAI-GOROMARU M, SAMEJIMA H and TAKAYANAGI I: The significant role of endothelin-3 in potentiating electrically stimulated contractions in the rat iris sphincter. *Gen Pharmacol* **28**: 365-369, 1997.
SHINKAI M, TSURUOKA H, WAKABAYASHI S, YAMAMOTO Y and TAKAYANAGI I: Pre- and postjunctional actions of endothelin in the rat iris sphincter preparation. *Naunyn Schmiedebergs Arch Pharmacol* **350**: 63-67, 1994.
SUDJARWO S A, HORI M, TAKAI M, URADE Y, OKADA T and KARAKI H: A novel subtype of endothelin B receptor mediating contraction in swine pulmonary vein. *Life Sci* **53**: 431-437, 1993.
SUDJARWO S A, HORI M, TANAKA T, MATSUDA Y, OKADA T and KARAKI H: Subtypes of endothelin ETA and ETB receptors mediating venous smooth muscle contraction. *Biochem Biophys Res Commun* **200**: 627-633, 1994.
UCHIKAWA Y, OKANO M, SAWADA A, ASADA Y, KOBAYASHI H, WADA A, NAO-I N, OHKURA M, TANAKA N and YAMAMOTO R: Relaxant effect of adrenomedullin on bovine isolated iris sphincter muscle under resting conditions. *Clin Exp Pharmacol Physiol* **32**: 675-680, 2005.

WIENCKE A K, NILSSON H, NIELSEN P J and NYBORG N C: Nonadrenergic noncholinergic vasodilation in bovine ciliary artery involves CGRP and neurogenic nitric oxide. *Invest Ophthalmol Vis Sci* **35**: 3268-3277, 1994.
YANAGISAWA M, INOUE A, TAKUWA Y, MITSUI Y, KOBAYASHI M and MASAKI T: The human preproendothelin-1 gene: possible regulation by endothelial phosphoinositide turnover signaling. *J Cardiovasc Pharmacol* **13 Suppl 5**: S13-17; discussion S18, 1989.
YANAGISAWA M, KURIHARA H, KIMURA S, GOTO K and MASAKI T: A novel peptide vasoconstrictor, endothelin, is produced by vascular endothelium and modulates smooth muscle Ca2+ channels. *J Hypertens Suppl* **6**: S188-191, 1988.
YONEYAMA T, HORI M, MAKATANI M, YAMAMURA T, TANAKA T, MATSUDA Y and KARAKI H: Subtypes of endothelin ETA and ETB receptors mediating tracheal smooth muscle contraction. *Biochem Biophys Res Commun* **207**: 668-674, 1995.
YORIO T, KRISHNAMOORTHY R and PRASANNA G: Endothelin: is it a contributor to glaucoma pathophysiology? *J Glaucoma* **11**: 259-270, 2002.
ZUCCARELLO M, BOCCALETTI R and RAPOPORT R M: Does blockade of endothelinB1-receptor activation increase endothelinB2/endothelinA receptor-mediated constriction in the rabbit basilar artery? *J Cardiovasc Pharmacol* **33**: 679-684, 1999.