ELSEVIER

European Journal of Pharmacology 378 (1999) 317–322

ejp

www.elsevier.nl/locate/ejphar

Betaxolol, a β_1-adrenoceptor antagonist, has an affinity for L-type Ca^{2+} channels

José Melena, John P.M. Wood, Neville N. Osborne *

Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford, OX2 6AW, UK

Received 6 May 1999; received in revised form 21 June 1999; accepted 25 June 1999

Abstract

The effect of betaxolol on the specific binding of [^{3}H]diltiazem and [^{3}H]nitrendipine to rat cortical membranes was examined. Betaxolol inhibited specific [^{3}H]diltiazem and [^{3}H]nitrendipine binding with IC_{50} values of 19.7 and 46.3 μM, respectively. The effect of betaxolol on L-type Ca^{2+} channels showed little stereospecificity, since similar inhibitions of radioligand binding were observed with both racemic betaxolol and L-betaxolol. The dissociation kinetics of [^{3}H]diltiazem were unaffected by 30 μM betaxolol, whereas it increased the [^{3}H]nitrendipine dissociation rate, thus suggesting that betaxolol directly interacts with the benzothiazepine binding site and allosterically modulates the dihydropyridine binding site. Carteolol, propranolol and timolol were also found to inhibit both specific [^{3}H]diltiazem and [^{3}H]nitrendipine binding to rat cortical membranes, but with less potency than betaxolol. The ability of betaxolol to interact with L-type Ca^{2+} channels may have a role in its therapeutic effects in the management of systemic hypertension and in reducing neuronal death as occurring in glaucoma.

Keywords: Betaxolol; Ca^{2+} channel, L-type; [^{3}H]Diltiazem; [^{3}H]Nitrendipine; β-Adrenoceptor antagonist

1. Introduction

β-Adrenoceptor antagonists are first-line agents for the treatment of systemic hypertension and glaucoma (Alward, 1998; Hennekens, 1998). Evidence obtained over the last few years suggests that some or all of the β-adrenoceptor antagonists may exert part of their therapeutic actions by reducing to a greater or lesser extent Ca^{2+} influx into cells through a mechanism not related with β-adrenoceptor blockade (Hester et al., 1994; Setoguchi et al., 1995; Hoste and Sys, 1998).

Betaxolol and propranolol have been reported to produce a relaxing effect on isolated blood vessels in a number of different species and preparations, whereas atenolol, carteolol and timolol generally showed a much weaker vascular relaxing action (Hoste et al., 1990; Bessho et al., 1991; Hester et al., 1994; Hoste and Sys, 1994, 1998; Yu et al., 1998). This direct vasodilator effect of betaxolol, a β_1-selective adrenoceptor antagonist, was reduced by an increased extracellular Ca^{2+} concentration, which suggests that this drug may block Ca^{2+} influx across the cell membrane (Bessho et al., 1991). Moreover, betaxolol, but not propranolol, has recently been found to inhibit Ca^{2+} channel currents in vascular smooth muscle cells as have been shown for both diltiazem and nifedipine (Setoguchi et al., 1995).

The aim of the present work was to investigate whether betaxolol may directly interact with L-type voltage-dependent Ca^{2+} channels by examining its effect on the binding of [^{3}H]diltiazem and [^{3}H]nitrendipine to rat cortical membranes. The effects of carteolol, propranolol and timolol were also assessed for comparative purposes.

2. Materials and methods

2.1. Membrane preparation

The cerebral cortex from Wistar rats (250–350 g) bred in our laboratory was dissected and homogenised in ice-cold 50 mM Tris–HCl buffer (pH 7.4 at 25°C). The homogenate was washed by means of three consecutive centrifugation cycles (48,000 × *g*, 10 min, 4°C) with intermittent resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM Tris–HCl buffer

* Corresponding author. Tel.: +44-1865-248996; fax: +44-1865-794508; E-mail: neville.osborne@eye.ox.ac.uk

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00459-8

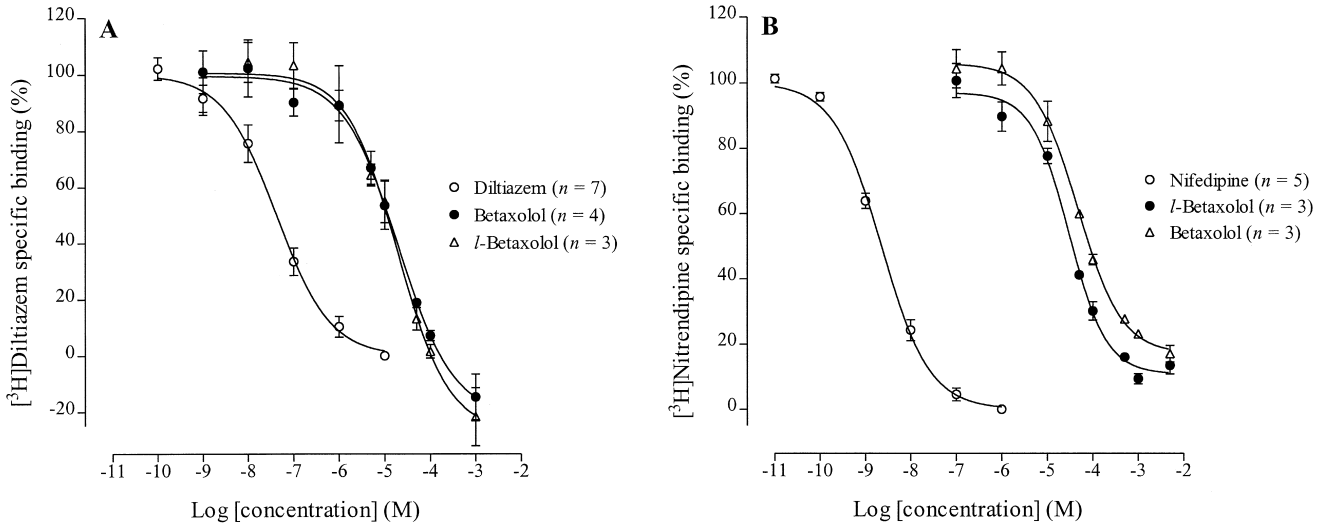

Fig. 1. (A) Effects of diltiazem, racemic betaxolol and L-betaxolol on [^{3}H]diltiazem (4 nM) specific binding to rat cortical membranes. Each point represents the mean ± S.E.M. of the number of experiments shown in parentheses, with each determination performed in duplicate. (B) Effects of nifedipine, racemic betaxolol and L-betaxolol on specific [^{3}H]nitrendipine (0.1 nM) binding to rat cortical membranes. Each point represents the mean ± S.E.M. of the number of experiments shown in parentheses performed in duplicate.

(pH 7.4) to yield an original tissue concentration of 200 mg/3 ml and stored at −70°C until use.

2.2. Radioligand binding

Radioligand binding studies with [^{3}H]diltiazem and [^{3}H]nitrendipine were performed as previously described (Mir and Spedding, 1987) with minor modifications. For [^{3}H]diltiazem competition binding studies, aliquots (200 μl) of the tissue homogenate (200 mg/3 ml) were incubated with 4 nM [^{3}H]diltiazem and various concentrations of the drugs tested in 50 mM Tris–HCl buffer (pH 7.4) in a final volume of 500 μl for 90 min at 25°C. At the end of the incubation, samples were diluted with 5 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4), immediately vacuum filtered through Whatman GF/B filters and washed twice with 5 ml of ice-cold buffer. The filters were presoaked in 0.1% polyethylenimine for 2 h to reduce radioactivity trapping on the filters. Under these washing conditions, the blank filter radioactivity was not displaced by any of the drugs studied. Radioactivity on the filters was measured by liquid scintillation spectrometry in 5 ml of Insta-gel Plus (Packard, Groningen, The Netherlands). Non-specific binding of [^{3}H]diltiazem was determined in the presence of 10 μM diltiazem and represented 25–35% of the total binding. Dissociation kinetics were measured by preincubating samples with 4 nM [^{3}H]diltiazem for 90 min at 25°C. Dissociation was induced by the addition of 10 μM unlabelled diltiazem and samples were filtered at various times in the presence or absence of 30 μM betaxolol.

For [^{3}H]nitrendipine competition binding studies, aliquots (200 μl) of 4-fold diluted tissue homogenate (50 mg/3 ml) were incubated with 0.1 nM [^{3}H]nitrendipine and various concentrations of the drugs tested in 50 mM Tris–HCl buffer (pH 7.4) with a final volume of 500 μl for 90 min at 25°C. At the end of incubation, samples were diluted with 5 ml of ice-cold buffer, filtered through Whatman GF/B filters and washed twice with 5 ml of ice-cold buffer. Radioactivity on the filters was measured as described above. Non-specific binding of [^{3}H]nitrendipine was determined in the presence of 1 μM nifedipine and represented 15–20% of the total binding. Dissociation kinetics were measured by preincubating samples with 0.1 nM [^{3}H]nitrendipine for 90 min at 25°C. An excess of unlabeled nifedipine (1 μM) was then added and samples were filtered at various times in the presence or absence of 30 μM betaxolol. These experiments were carried out in subdued light to minimise [^{3}H]nitrendipine and nifedipine degradation.

2.3. Data analysis

The slope factors and IC_{50} values for competition binding data were obtained using a nonlinear method (Graph-

Table 1
Effects of betaxolol on dissociation rate constant (K_{-1}) of [^{3}H]diltiazem and [^{3}H]nitrendipine from rat cortical membranes
K_{-1} values are the mean ± S.E.M. of n experiments done in duplicate.

	n	K_{-1} (min^{-1})	r^b
[^{3}H]Diltiazem			
Control (diltiazem 10 μM)	5	0.0424 ± 0.0037	0.97
+ Betaxolol (30 μM)	5	0.0490 ± 0.0055	0.95
[^{3}H]Nitrendipine			
Control (nifedipine 1 μM)	3	0.0473 ± 0.0012	1.00
+ Betaxolol (30 μM)	3	0.0778 ± 0.0014[a]	1.00

[a] Significantly different from control by unpaired t-test ($P < 0.001$).
[b] Linear regression coefficient.

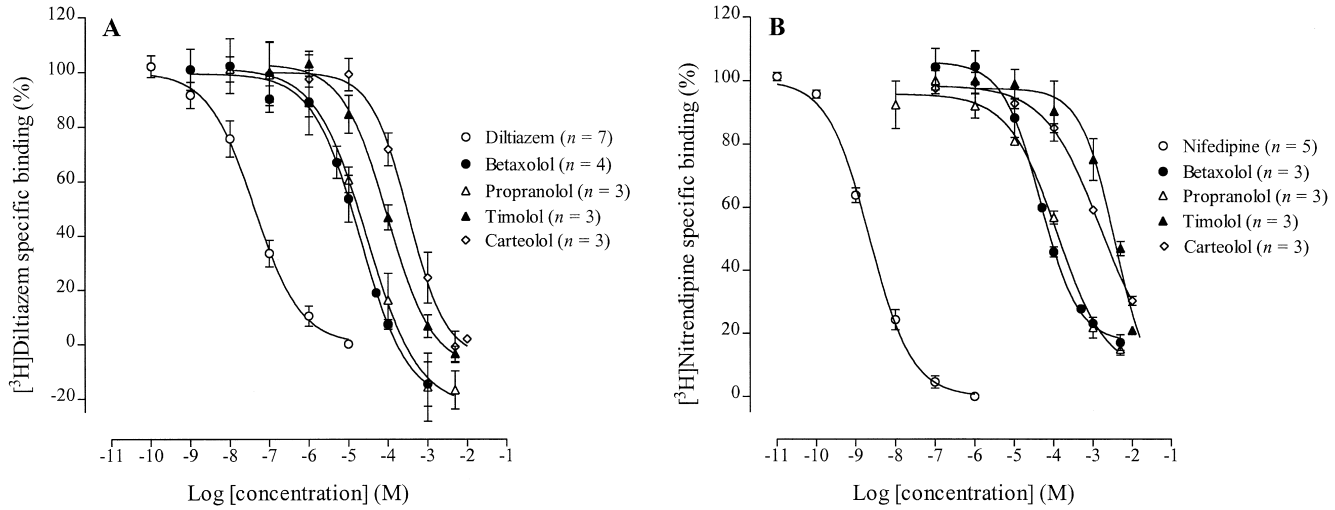

Fig. 2. Effects of betaxolol, carteolol, propranolol and timolol on the specific binding of 4 nM [^{3}H]diltiazem (A) and 0.1 nM [^{3}H]nitrendipine (B) to rat cortical membranes. Effects of diltiazem and nifedipine on specific [^{3}H]diltiazem (A) and [^{3}H]nitrendipine (B) binding, respectively, are also shown for comparison. Each point represents the mean ± S.E.M. of the number of experiments shown in parentheses performed in duplicate.

Pad Prism 1.0). The dissociation rate constant (K_{-1}) was determined from linear regression analysis of ln B_t/B_0 vs. time, where B_t and B_0 were the amount of radioligand bound at t and zero time, respectively.

All experiments were performed in duplicate and data are expressed as mean ± S.E.M. Statistical analysis were performed by using an unpaired Student's t-test and One-way analysis of variance using the Bonferroni post-test. Values of $P < 0.05$ were considered statistically significant.

2.4. Materials

D-*cis*-[^{3}H]Diltiazem (85.5 Ci/mmol) and [^{3}H]nitrendipine (87.0 Ci/mmol) were purchased from New England Nuclear (Stevenage, UK). Diltiazem hydrochloride, nifedipine, (*S*)-L-propranolol hydrochloride and timolol maleate all came from Sigma (Poole, UK). Betaxolol and L-betaxolol hydrochlorides were supplied by Alcon Research Laboratories (Fort Worth, TX, USA). Teoptic® (CIBA Vision Ophthalmics, Hedge End, UK) was used as source of carteolol hydrochloride.

3. Results

Fig. 1 shows the effects of racemic betaxolol and L-betaxolol on the binding of [^{3}H]diltiazem and [^{3}H]-nitrendipine to rat cortical membranes. Specific binding of [^{3}H]diltiazem to cortical membranes was completely displaced by racemic betaxolol and L-betaxolol in a concentration-dependent fashion. Racemic betaxolol inhibited [^{3}H]diltiazem binding with an IC_{50} of 19.7 μM ($-\log IC_{50} = 4.71 \pm 0.08$) and a slope factor of 0.73 ± 0.08 ($n = 4$), whereas the calculated IC_{50} and slope factor values for L-betaxolol were 19.2 μM ($-\log IC_{50} = 4.72 \pm 0.10$) and 0.78 ± 0.11 ($n = 3$), respectively. Diltiazem competitively inhibited [^{3}H]diltiazem binding with an IC_{50} of 42.1 nM ($-\log IC_{50} = 7.38 \pm 0.05$, slope factor = 0.74 ± 0.05, $n = 7$). Both racemic betaxolol and L-betaxolol at high concentrations appeared to cause a displacement of about 20–25% in the non-specific [^{3}H]diltiazem binding to rat cortical membranes. Such displacements were not due to any reduction in radioactivity attached to filters and similar non-specific binding inhibitions were observed with equimolar concentrations of diltiazem.

Specific binding of [^{3}H]nitrendipine was almost completely inhibited by racemic betaxolol (83%) and L-betaxolol (89%) in a concentration dependent manner. Betaxolol displaced specific [^{3}H]nitrendipine binding with an IC_{50} of 46.3 μM ($-\log IC_{50} = 4.33 \pm 0.02$) and a slope factor of 0.90 ± 0.06 ($n = 3$), while values obtained for L-betaxolol showed no statistically significant differences (IC_{50} = 29.5 μM, $-\log IC_{50} = 4.53 \pm 0.08$, slope factor = 1.05 ± 0.18, $n = 3$). Nifedipine competitively inhibited [^{3}H]nitrendipine binding with an IC_{50} of 2.3 nM ($-\log IC_{50} = 8.65 \pm 0.04$, slope factor = 0.82 ± 0.06, $n = 5$).

Table 2
IC_{50} values and slope factors for inhibition of [^{3}H]diltiazem specific binding to rat cortical membranes
−Log IC_{50} values and slope factors are the mean ± S.E.M. of the number of experiments shown in parentheses done in duplicate.

Drug	IC_{50} (μM)	−Log IC_{50}	Slope factor
Diltiazem ($n = 7$)	0.0421	7.38 ± 0.05	0.74 ± 0.05
Betaxolol ($n = 4$)	19.7	4.71 ± 0.08	0.73 ± 0.08
(*S*)-L-Propranolol ($n = 3$)	29.2	4.53 ± 0.07	0.67 ± 0.06
Timolol ($n = 3$)	96.0	4.02 ± 0.09[a]	0.77 ± 0.12
Carteolol ($n = 3$)	327.0	3.49 ± 0.10[a]	0.91 ± 0.15

[a] Significantly different from betaxolol IC_{50} value by One-way ANOVA ($P < 0.001$).

Table 3
IC_{50} values and slope factors for inhibition of [^{3}H]nitrendipine specific binding to rat cortical membranes
$-\text{Log } IC_{50}$ values and slope factors are the mean ± S.E.M. of the number of experiments shown in parentheses done in duplicate.

Drug	IC_{50} (μM)	$-\text{Log } IC_{50}$	Slope factor
Nifedipine ($n=5$)	0.0023	8.65 ± 0.04	0.82 ± 0.06
Betaxolol ($n=3$)	46.3	4.33 ± 0.02	0.90 ± 0.06
(*S*)-L-Propranolol ($n=3$)	123.0	3.91 ± 0.18	0.70 ± 0.17
Timolol ($n=3$)	3653.7	2.44 ± 0.08[a]	0.99 ± 0.20
Carteolol ($n=3$)	1516.3	2.82 ± 0.24[a]	0.62 ± 0.15

[a] Significantly different from betaxolol IC_{50} value by One-way ANOVA ($P < 0.001$).

The effects of betaxolol on the dissociation kinetics of [^{3}H]diltiazem and [^{3}H]nitrendipine were assessed to further clarify the mechanism of betaxolol interaction with these two binding sites on the L-type Ca^{2+} channel (Table 1). Betaxolol was found to significantly increase the dissociation rate of [^{3}H]nitrendipine, but not to affect that of [^{3}H]diltiazem.

The effects of other β-adrenoceptor antagonists, such as (*S*)-L-propranolol, timolol and carteolol, on the specific binding of [^{3}H]diltiazem and [^{3}H]nitrendipine to rat cortical membranes were also examined. (*S*)-L-Propranolol, timolol and carteolol were found to completely displace the specific binding of [^{3}H]diltiazem in a concentration-related fashion (Fig. 2), with IC_{50} values and slope factors shown in Table 2. Betaxolol was about 1.5, 5 and 16 times more potent in inhibiting [^{3}H]diltiazem binding than propranolol, timolol and carteolol, respectively. Like betaxolol, propranolol at high concentrations also partially displaced the non-specific binding of [^{3}H]diltiazem. As shown in Fig. 2, (*S*)-L-propranolol, timolol and carteolol produced a concentration-dependent inhibition of [^{3}H]nitrendipine binding to rat cortical membranes. IC_{50} values in Table 3 indicate that betaxolol was about 2.5, 33 and 79 times more potent than propranolol, carteolol and timolol, respectively.

4. Discussion

In the present study, betaxolol was found to interact both with 1,5-benzothiazepine (diltiazem) and 1,4-dihydropyridine (nitrendipine) binding sites on L-type Ca^{2+} channels of rat cortical membranes. Betaxolol inhibited [^{3}H]diltiazem and [^{3}H]nitrendipine binding to cortical membranes with IC_{50} values of 19.7 and 46.3 μM, respectively. In addition, the IC_{50} values obtained for diltiazem (42.1 nM) and nifedipine (2.3 nM) were in close agreement with those previously reported in this tissue (Schoemaker and Langer, 1985; Mir and Spedding, 1987; Schaeffer et al., 1991). The effect of betaxolol on the specific binding of both [^{3}H]diltiazem and [^{3}H]nitrendipine showed no stereoselectivity, since similar inhibitions were observed with either racemic betaxolol or L-betaxolol. Results from competition experiments suggest that betaxolol directly interacts with either the benzothiazepine or the dihydropyridine site associated with L-type Ca^{2+} channels, since slope factors approximated unity in both cases. According to Ehlert (1988), however, if the magnitude of negative heterotropic cooperativity is large, an allosteric modulator is likely to inhibit the specific binding of a radioligand in an apparently competitive fashion and therefore studies of dissociation kinetics are needed to clarify interactions with the binding site. Compounds which allosterically modulate radioligand binding are expected to alter dissociation kinetics, whereas those that directly compete with radioligands for the same binding site are expected not to modify dissociation rates. In the present study, betaxolol showed no effect on the dissociation kinetics of [^{3}H]diltiazem, whereas it increased the dissociation rate of [^{3}H]nitrendipine. Such data indicate that betaxolol directly interacts with the benzothiazepine site on the L-type Ca^{2+} channel and modulates the binding to the dihydropyridine site by negative heterotropic allostery. This pharmacological profile resembles that of L-*cis*-diltiazem, which has been reported to increase the dihydropyridine dissociation, while D-*cis*-diltiazem produces the opposite effect (Ikeda et al., 1991).

The other β-adrenoceptor antagonists tested in our study, carteolol, propranolol and timolol, also inhibited the specific binding of [^{3}H]diltiazem and [^{3}H]nitrendipine, although all were less potent than betaxolol. Carteolol, propranolol and timolol were found to completely displace the binding of [^{3}H]diltiazem to rat cortical membranes, but only propranolol exhibited an IC_{50} value close to that of betaxolol, with IC_{50} values for timolol and carteolol being about 5 and 15 times greater, respectively. Such differences were even more at the [^{3}H]nitrendipine binding site on the L-type Ca^{2+} channel, where propranolol, carteolol and timolol were about 2.5, 33 and 79 times less potent than betaxolol, respectively.

These radioligand binding data concerning the interaction of betaxolol, propranolol, carteolol and timolol with L-type Ca^{2+} channels are consistent with previous evidence describing the direct vascular relaxing action of these drugs. Betaxolol has been found to exert a concentration-dependent relaxing effect in rat aortic, renal, mesenteric and femoral strips with EC_{50} values in the 22–48 μM range (Bessho et al., 1991), very similar to IC_{50} values for the inhibition of both [^{3}H]diltiazem and [^{3}H]nitrendipine binding reported in this study. In bovine retinal microarteries, which lack adrenergic nerves and β-adrenoceptors, both betaxolol and propranolol have been shown to equally relax K^+-induced contractions at micromolar concentrations, higher doses of timolol being needed to obtain a weak relaxing effect (Hoste et al., 1990; Hoste and Sys, 1994, 1998). Accordingly, betaxolol has been reported to be at least 1000 times more potent than timolol in relaxing pig retinal arterioles pre-contracted with endothelin-1

(Yu et al., 1998). The vascular relaxing effect of betaxolol, carteolol and timolol has been directly compared in both porcine long posterior ciliary arteries and rabbit external iliac arteries (Hester et al., 1994). In this study, betaxolol relaxed K^+-contracted porcine arteries with an EC_{50} value of 100 μM, exhibiting 6-fold and 10-fold more activity than carteolol and timolol, respectively. However, rabbit arteries showed less sensitivity to betaxolol (EC_{50} = 448 μM) and even less to timolol and carteolol, whose potencies were 25 and 63 times lower, respectively, than that of betaxolol in this tissue. In general, the present radioligand binding data concerning the interaction of betaxolol, propranolol, carteolol and timolol with L-type Ca^{2+} channels are in close agreement with their reported concentrations required to produce a direct vascular relaxing effect. In this regard, it should be borne in mind that the α_1-subunit ($\alpha_{1C\text{-}a}$) splice variant in vascular L-type Ca^{2+} channels is more sensitive to dihydropyridines than to benzothiazepines (Welling et al., 1997). Therefore, it is likely that effects of β-adrenoceptor antagonists on specific [^{3}H]nitrendipine binding resemble more closely the vascular actions of these drugs.

The results reported here and elsewhere strongly support the hypothesis that the relaxing effect of some β-adrenoceptor antagonists on vascular vessels results from inhibition of Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels. Betaxolol has been found to produce a concentration-related rightward parallel shift of the concentration–response curve for Ca^{2+} both in K^+-depolarized rat aorta and porcine long posterior ciliary arteries, an action very similar to that elicited by diltiazem (Bessho et al., 1991; Hester et al., 1994). Cytosolic Ca^{2+} concentrations measured with fura-2 fluorescence were also reduced by betaxolol in K^+-contracted rat aortic strips (Bessho et al., 1991). This effect was antagonised by increased external Ca^{2+} concentrations. Similarly, betaxolol has been reported to inhibit Ca^{2+} channel currents recorded in single smooth muscle cells from guinea-pig mesenteric artery and portal vein with EC_{50} values of 45 and 46 μM (Setoguchi et al., 1995), nearly identical to that obtained here for the inhibition of [^{3}H]nitrendipine binding. Nevertheless, 10 μM propranolol did not inhibit these Ca^{2+} currents, although, according to our radioligand binding data, higher concentrations of propranolol would have been required to rule out any inhibitory effect of this drug on Ca^{2+} currents. In this study (Setoguchi et al., 1995), the two isomers of betaxolol, D- and L-betaxolol, had the same potency in inhibiting Ca^{2+} channel currents, which clearly agrees with our radioligand binding data, where racemic betaxolol and L-betaxolol were equally effective in displacing the specific binding of [^{3}H]diltiazem and [^{3}H]nitrendipine. In contrast, the β-adrenoceptor blocking action of betaxolol shows high stereospecificity (Nathanson, 1988).

This potential L-type Ca^{2+} channel-blocking activity of betaxolol and, at higher concentrations, of other β-adrenoceptor antagonists may be responsible for the long-term reduction of peripheral resistance produced by these drugs. Intravenous betaxolol has even been reported to decrease peripheral resistance in anaesthetised dogs (Satoh et al., 1990) and hypertensive rats (Bessho et al., 1990), which may result from a direct interaction of this drug with L-type Ca^{2+} channels. The antihypertensive effects of betaxolol and propranolol are observed at plasma concentrations of 0.10–0.15 μM, but, at least for propranolol, a high inter-individual variability exists and serum levels above 1 μM are found in some patients (Walle et al., 1985; Beresford and Heel, 1986). Although these plasma concentrations appear relatively high as compared with those required to displace [^{3}H]diltiazem and [^{3}H]nitrendipine binding, it should be noted that, as a result of their high lipophilicity, both betaxolol and propranolol may accumulate in the cell membrane when administered over long term, as do other lipophilic drugs. It has been shown that concentrations of some lipophilic drugs, including propranolol and dihydropyridines, are substantially higher (over 3 orders of magnitude) in the membrane bilayer than in the extra-membrane, aqueous surroundings (Mason et al., 1991). Accordingly, betaxolol and propranolol could reach concentrations in the cell membrane high enough to inhibit L-type Ca^{2+} chanels.

The ability of betaxolol to interact with L-type Ca^{2+} channels may also account for some of its ocular actions. Despite timolol being more effective than betaxolol in lowering intraocular pressure, it has been shown that long-term treatment of betaxolol tends to have more beneficial effects on glaucomatous visual fields losses than timolol (Messmer et al., 1991; Kaiser et al., 1992; Collignon-Brach, 1994; Kaiser et al., 1994; Drance, 1998). These findings could be ascribed either to a direct neuroprotective action of betaxolol or a betaxolol-induced increase in ocular blood flow. In this regard, long-term topical betaxolol has been reported to increase tissue blood flow velocity in the rabbit optic nerve head in a similar way to nilvadipine, a dihydropyridine Ca^{2+} channel blocker (Araie and Muta, 1997). Evidence also exists to suggest that betaxolol is a retinal neuroprotective agent (Osborne et al., 1997, 1999). Betaxolol was found to reduce both the kainate-induced increase in intracellular Ca^{2+} concentration in chick retinal cultures (Osborne et al., 1997) and the NMDA-induced influx of Ca^{2+} into cortical neurones (Osborne et al., 1999). Both results were probably due to a blockade of Ca^{2+} influx via voltage-dependent channels (Osborne et al., 1997, 1999). Thus, the Ca^{2+} channel-blocking action of betaxolol may play a role both in its neuroprotective and ocular blood flow-increasing effect.

In summary, the present data show that betaxolol interacts with L-type Ca^{2+} channels. Evidence from dissociation kinetics suggests that betaxolol directly competes for the benzothiazepine binding site, whereas it allosterically modulates the dihydropyridine site. This ability of betaxolol to modulate L-type Ca^{2+} channels and so reduce

influx of Ca^{2+} may be responsible for some of the effects of this drug on blood vessels when used for the treatment of systemic hypertension as well as on neurons to protect against excessive influx of Ca^{2+} as would occur in ischaemia and possibly glaucoma.

Acknowledgements

J.M. research was supported by a post-doctoral Marie Curie grant (TMR programme, European Commission). We are also grateful to Dr. L. DeSantis of Alcon Research Laboratories (Fort Worth, TX, USA) for providing scholarly and financial support.

References

Alward, W.L., 1998. Drug therapy: medical management of glaucoma. New Engl. J. Med. 339, 1298–1307.

Araie, M., Muta, K., 1997. Effect of long-term topical betaxolol on tissue circulation in the iris and optic nerve head. Exp. Eye Res. 64, 167–172.

Beresford, R., Heel, R.C., 1986. Betaxolol: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in hypertension. Drugs 31, 6–28.

Bessho, H., Suzuki, J., Narimatsu, A., Tobe, A., 1990. Antihypertensive effect of betaxolol, a cardioselective β-adrenoceptor antagonist, in experimental hypertensive rats. Jpn. J. Pharmacol. 52 (Suppl. 1), 315P.

Bessho, H., Suzuki, J., Tobe, A., 1991. Vascular effects of betaxolol, a cardioselective β-adrenoceptor antagonist, in isolated rat arteries. Jpn. J. Pharmacol. 55, 351–358.

Collignon-Brach, J., 1994. Longterm effect of topical beta-blockers on intraocular pressure and visual field sensitivity in ocular hypertension and chronic open-angle glaucoma. Surv. Ophthalmol. 38, S149–155, suppl.

Drance, S.M., 1998. A comparison of the effects of betaxolol, timolol and pilocarpine on visual function in patients with open-angle glaucoma. J. Glaucoma 7, 247–252.

Ehlert, F.J., 1988. Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods. Mol. Pharmacol. 33, 187–194.

Hennekens, C.H., 1998. Lessons from hypertension trials. Am. J. Med. 104, 50S–53S.

Hester, R.K., Chen, Z., Becker, E.J., McLaughlin, M., DeSantis, L., 1994. The direct vascular relaxing action of betaxolol, carteolol and timolol in porcine long posterior ciliary artery. Surv. Ophthalmol. 38, S125–134, suppl.

Hoste, A.M., Sys, S.U., 1994. The relaxant action of betaxolol on isolated bovine retinal microarteries. Curr. Eye Res. 13, 483–487.

Hoste, A.M., Sys, S.U., 1998. Ca^{2+} channel-blocking activity of propranolol and betaxolol in isolated bovine retinal microartery. J. Cardiovasc. Pharmacol. 32, 390–396.

Hoste, A.M., Boels, P.J., Andries, L.J., Brutsaert, D.L., De Laey, J.J., 1990. Effects of beta-antagonists on contraction of bovine retinal microarteries in vitro. Invest. Ophthalmol. Vis. Sci. 31, 1231–1237.

Ikeda, S., Oka, J.-I., Nagao, T., 1991. Effects of four diltiazem stereoisomers on binding of d-cis-[^{3}H]diltiazem and (+)-[^{3}H]PN200-110 to rabbit T-tubule calcium channels. Eur. J. Pharmacol. 208, 199–205.

Kaiser, H.J., Flammer, J., Messmer, C., Stümpfig, D., Hendrickson, P., 1992. Thirty-month visual field follow-up of glaucoma patients treated with beta-blockers. J. Glaucoma 1, 153–155.

Kaiser, H.J., Flammer, J., Stümpfig, D., Hendrickson, P., 1994. Longterm visual field follow-up of glaucoma patients treated with beta-blockers. Surv. Ophthalmol. 38, S156–159, suppl.

Mason, R.C., Rhodes, D.G., Herbette, L.G., 1991. Reevaluating equilibrium and kinetic binding parameters for lipophilic drugs based on a structural model for drug interaction with biological membranes. J. Med. Chem. 34, 869–877.

Messmer, C., Flammer, J., Stümpfig, D., 1991. Influence of betaxolol and timolol on the visual fields of patients with glaucoma. Am. J. Ophthalmol. 112, 678–681.

Mir, K.A., Spedding, M., 1987. Calcium antagonist properties of diclofurime isomers: II. Molecular aspects: allosteric interactions with dihydropyridine recognition sites. J. Cardiovasc. Pharmacol. 9, 469–477.

Nathanson, J.A., 1988. Stereospecificiy of *beta* adrenergic antagonists: R-enantiomers show increased selectivity for *beta*-2 receptors in ciliary process. J. Pharmacol. Exp. Ther. 245, 94–101.

Osborne, N.N., Cazevieille, C., Carvalho, A.L., Larsen, A.K., DeSantis, L., 1997. In vivo and in vitro experiments show that betaxolol is a retinal neuroprotective agent. Brain Res. 751, 113–123.

Osborne, N.N., DeSantis, L., Bae, J.H., Ugarte, M., Wood, J.P.M., Chidlow, G., 1999. Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischaemia to the rat retina. Exp. Eye Res., in press.

Satoh, N., Suzuki, J., Bessho, H., Kitada, Y., Narimatsu, A., Tobe, A., 1990. Effects of betaxolol on cardiohemodynamics and coronary circulation in anesthetized dogs: comparison with atenolol and propranolol. Jpn. J. Pharmacol. 54, 113–119.

Schaeffer, P., Lugnier, C., Stoclet, J.-C., 1991. Interactions of calmodulin antagonists with calcium antagonists binding sites. Eur. J. Pharmacol. 206, 325–332.

Schoemaker, H., Langer, S.Z., 1985. [^{3}H]diltiazem binding to calcium channel antagonists recognition sites in rat cerebral cortex. Eur. J. Pharmacol. 111, 273–277.

Setoguchi, M., Ohya, Y., Abe, I., Fujishima, M., 1995. Inhibitory action of betaxolol, a β_1-selective adrenoceptor antagonist, on voltage-dependent calcium channels in guinea-pig artery and vein. Br. J. Pharmacol. 115, 198–202.

Walle, T., Byington, R.P., Furberg, C.D., McIntyre, K.M., Vokonas, P.S., 1985. Biologic determinants of propranolol disposition: results from 1308 patients in the beta-blocker heart attack trial. Clin. Pharmacol. Ther. 38, 509–518.

Welling, A., Ludwig, A., Zimmer, S., Klugbauer, N., Flockerzi, V., Hofmann, F., 1997. Alternatively spliced IS6 segments of the α_{1C} gene determine the tissue-specific dihydropyridine sensitivity of cardiac and vascular smooth muscle L-type Ca^{2+} channels. Circ. Res. 81, 526–532.

Yu, D.-Y., Su, E.-N., Cringle, S.J., Alder, V.A., Yu, P.K., DeSantis, L., 1998. Effect of betaxolol, timolol and nimodipine in pig retinal arterioles. Exp. Eye Res. 67, 73–81.