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# β-Adrenoceptor antagonist activities and binding affinities of timolol enantiomers in rat atria

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**Abstract**—*S*-Timolol is an effective anti-glaucoma drug, but has potentially hazardous side effects. Recently, *R*-timolol, also, has been reported to be effective in lowering elevated intraocular pressure. In the present study, the β-adrenoceptor antagonist activities and binding of *R*- and *S*-enantiomers of timolol have been examined on rat atrial preparations. The β-antagonistic activities were investigated using spontaneously beating rat heart atria. Both timolol enantiomers inhibited (-)-isoprenaline-induced chronotropic action competitively. *S*-Timolol was about 54 times more potent than *R*-timolol. The apparent binding affinities of timolol enantiomers to $\beta_1$- and $\beta_2$-adrenoceptors were determined by a radioligand binding assay using (-)-[$^{125}$I]iodocyanopindolol (ICYP) as a marker and CGP 20712 A as a $\beta_1$- and ICI 118,551 as a $\beta_2$-adrenoceptor antagonist. Both enantiomers of timolol inhibited ICYP binding in nanomolar concentrations with Hill coefficients near unity. Neither enantiomer showed selectivity between $\beta_1$- and $\beta_2$-adrenoceptors, but *R*-timolol was approximately 30 times less active than *S*-timolol. It is concluded that *R*-timolol is a relatively potent non-selective β-adrenoceptor blocking agent, but may possibly exert a more localized β-adrenoceptor action in the eye than *S*-timolol, thus improving the safety of ocular timolol therapy.

*S*-Timolol is a potent nonselective β-adrenoceptor antagonist. In addition to its cardiovascular indications, it is widely used in the treatment of glaucoma in the form of eyedrops, as an efficient intraocular pressure (IOP) lowering agent. However, substantial amounts of timolol can be absorbed systemically after its topical application (Zimmerman et al 1984; Kaila et al 1985), and cardiovascular, pulmonary and central nervous side effects have been described after timolol eyedrops (Noyes & Chervinsky 1980; Østergaard Laursen & Bjerrum 1982; Van Buskirk & Fraunfelder 1984).

Recently, the *R*-isomer of timolol in the form of 1% eyedrops, has been found to be effective in lowering elevated IOP when applied topically to the eye (Keates & Stone 1984), although the receptor binding and biological effects of the *R*-isomers of betablocking agents are generally considered to be lower than those of the *S*-isomers. Consequently, the use of the *R*-isomer in timolol eyedrops might reduce the side effects following systemic betablockade.

The present study was performed to characterize and quantitate the β-adrenoceptor blocking activities and binding affinities of the two optical isomers of timolol in rat atria.

## Materials and methods

*Spontaneously beating rat atria.* Spontaneously beating rat atria from male Wistar rats (250–350 g) were used. The rats were pretreated with reserpine 5 mg kg$^{-1}$ s.c. 16–24 h before death to deplete endogenous noradrenaline stores and were then killed with a blow to the neck. The hearts were rapidly removed and placed in Krebs-Henseleit solution (composition in mmol L$^{-1}$: NaCl 120, KCl 5·5, $CaCl_2$ 2·5, $NaH_2PO_4$ 1·2, $MgCl_2$ 1·2, (+)-glucose 11, $CaNa_2EDTA$ 0·029) equilibrated with 95% $O_2$ – 5% $CO_2$ and maintained at 37°C. The atria were dissected free of ventricular and other surrounding tissues, suspended in an organ bath and connected to a Grass FT.03 force displacement transducer. A resting tension of 500 mg was maintained. The spontaneous contractions were displayed on a Grass 79 D polygraph and the beating rate was recorded by means of a cardiotachograph. The atria were allowed to stabilize for 10–20 min and then exposed for 10 min to 5 μmol L$^{-1}$ phenoxybenzamine to block α-adrenoceptors. The tissues were then washed three times and challenged using 10 nmol L$^{-1}$ isoprenaline, followed by washing again until the responses returned to baseline. Two additional washes were further applied at a 5 min interval and atria were then equilibrated for 30 min before generating dose-response curves for isoprenaline.

The $pA_2$ values for both *R*- and *S*-timomol were determined by the method of Arunlakshana & Schild (1959). A dose-response curve for isoprenaline was first generated, three increasing concentrations of the antagonist were then added, and after each antagonist concentration the isoprenaline dose-response curve was repeated. The responses were measured as a percentage of the maximal increase in the beating rate of the atria. The EC50 values for each dose-response curve were calculated by linear regression analysis of all points between 20% and 80% of the maximal response to isoprenaline.

*Measurement of (-)-[$^{125}$I]iodocyanopindolol (ICYP) binding.* The binding assay was a modification of the method described by Juberg et al (1985). The right and left atrial tissues of twelve rats were homogenized in Tris-HCl (10 mmol L$^{-1}$) buffer containing 154 mmol L$^{-1}$ NaCl. the homogenate was centrifuged at 20 000 *g* for 20 min at 4°C and washed by resuspending the pellet in the buffer and recentrifuging the membrane. Saturation isotherms of equilibrium binding were determined using ten ICYP concentrations (from about 5 pmol L$^{-1}$ to 500 nmol L$^{-1}$) and then analysed by unweighted linear regression. Non-specific binding was determined in the presence of 10 μmol L$^{-1}$ (-)-isoprenaline.

*Blockade of $\beta_1$- or $\beta_2$-adrenoceptors in cardiac membranes.* $\beta_1$-adrenoceptors were blocked by using the highly $\beta_1$-selective antagonist CGP 20712 A (Dooley et al 1986) in a concentration (900 nmol L$^{-1}$) which inhibited specific ICYP binding by 70–75%. $\beta_2$-adrenoceptors were blocked with the highly $\beta_2$-selective antagonist ICI 118,551 (Bilski et al 1983) in a concentration (250 nmol L$^{-1}$) which inhibited specific ICYP binding by 25–30%. The ability of timolol enantiomers to compete for ICYP (90 pmol L$^{-1}$) binding at $\beta_1$- or $\beta_2$-adrenoceptor binding sites was determined by incubation with 10–11 concentrations of both enantiomers. The $K_i$ values (apparent affinities) and Hill coefficients were calculated with a computer programme (LIGAND) by fitting the inhibition curves using non-linear regression and minimizing the sum of squares of the differences (McPherson 1985).

*Drugs. R*- and *S*-Timolol were kindly donated by Star Pharmaceuticals (Finland), ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) by ICI Pharmaceuticals (UK), CGP 20712 A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1H-imidazole-2-yl)-phenoxy)propyl)amino)-ethoxy)-benzamide) by Ciba-Geigy (Switzerland) and phenoxybenzamine HCl by Smith, Kline and French, UK). The other drugs were purchased from the following sources: Reser-

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Table 1. Comparison of *S*- and *R*-timolol. $pA_2$ values obtained from competitive antagonism of isoprenaline-induced increases in rate of spontaneously beating rat atria (means ± s.e.m. of five determinations) and inhibition constants and Hill coefficients determined by the ICYP binding assay (means ± s.e.m. of three determinations).

| | | *S*-Timolol | *R*-Timolol | potency ratio (*S*/*R*) |
|---|---|---|---|---|
| | $pA_2$ | 9·16 ± 0·04 | 7·43 ± 0·04 | 54 |
| | slope | 1·10 ± 0·04 | 1·06 ± 0·03 | |
| $\beta_1$- | $K_i$ | 0·94 ± 0·02 nM | 28·8 ± 2·02 nM | 30 |
| | Hill coeff. | 0·95 ± 0·04 | 0·99 ± 0·04 | |
| $\beta_2$- | $K_i$ | 0·60 ± 0·08 nM | 18·1 ± 3·11 nM | 31 |
| | Hill coeff. | 0·89 ± 0·05 | 0·94 ± 0·05 | |

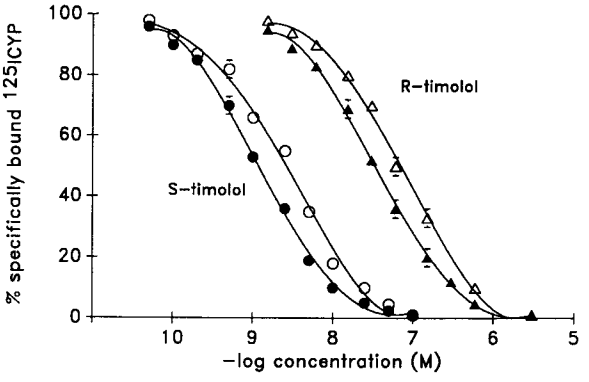


FIG. 1. Inhibition of specific $^{125}$ICYP binding by *S*-timolol (circles) or *R*-timolol (triangles) in the presence of 250 nmol $L^{-1}$ ICI 118,551, a selective $\beta_2$-adrenoceptor antagonist ($\beta_1$-binding, open symbols) or 900 nmol $L^{-1}$ CGP 20712 A, a selective $\beta_1$-adrenoceptor antagonist ($\beta_2$-binding, filled symbols). Each point represents the mean ± s.e.m. of three determinations.

pine (Serpasil, Ciba-Geigy, Switzerland); (-)-isoprenaline-HCl (Sigma, USA); (-)-iodocyanopindolol (DuPont, FRG).

## Results

Neither antagonist affected the basal beating rates nor the maximal responses to isoprenaline at any concentration used. Both timolol enantiomers inhibited isoprenaline-induced positive chronotropic action in a competitive manner. The *S*-enantiomer of timolol showed 54 times greater potency than the *R*-isomer in inhibiting isoprenaline induced tachycardia. Both enantiomers had the slopes of Schild plots near unity (Table 1).

In the binding studies, a dissociation constant ($K_d$) of 31·9 ± 1·0 pmol $L^{-1}$ (mean ± s.e.m., four determinations) was calculated for ICYP from the saturation isotherms. Both enantiomers inhibited ICYP binding competitively with Hill coefficients near unity. The *S*-isomer showed about 30 times greater affinity for both $\beta_1$- and $\beta_2$-adrenoceptors than the *R*-isomer (Fig. 1, Table 1).

## Discussion

The results indicate that *R*-timolol exerts a marked $\beta_1$-blocking action in spontaneously beating rat atria, although it is 54 times less potent than *S*-timolol. It also binds with a high affinity to both $\beta_1$- and $\beta_2$-adrenoceptors, the ratios between the enantiomers being approximately 30. The $pA_2$ values correlate well with the corresponding binding affinities to $\beta_1$-adrenoceptors. The method of measuring biological response, however, seems to be more discriminating than the receptor binding method, since the difference between the enantiomers is larger when calculated from the $pA_2$ values than from the $K_i$ values. Similar results have been reported earlier by Juberg et al (1985), who found potency ratios of 105 and 65 in biological and receptor binding experiments, respectively.

Both *R*-timolol and *S*-timolol are non-selective $\beta$-blocking agents, since neither enantiomer exhibited a clear selectivity towards the subtypes of $\beta$-adrenoceptors in the binding assays. The binding affinity of both enantiomers for $\beta_2$-adrenoceptors was about double that for $\beta_1$-adrenoceptors.

Since *R*-timolol has been thought to be almost inactive in blocking $\beta$-adrenoceptors it has been suggested that the mechanism(s?) mediating the ocular hypotensive effect of timolol could be independent of $\beta$-adrenoceptors (Keates & Stone 1984). However, concentrations of *S*-timolol eye drops used in the treatment of glaucoma are usually 0·25–0·5% whereas *R*-timomol has been found to be effective as a 1% solution. In addition, ocular timolol concentrations are high after topical application (Huupponen et al 1987). Nathanson (1988) has recently claimed that $\beta$-adrenergic antagonists demonstrate much less stereoselectivity in the rabbit ciliary process than they do in the rabbit heart. For example, *R*- and *S*-enantiomers of timolol were nearly equipotent in blocking isoprenaline-stimulated adenylate cyclase activation in ciliary body whereas in heart *S*-timolol was 44 times more potent than *R*-timolol. Thus, *R*-timolol may possibly exert a more localized $\beta$-adrenoceptor antagonist action improving the safety of ocular timolol therapy.

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