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Autonomic regulation involved in the ocular hypotensive action of β-adrenergic blocking agents

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The effects of racemic propranolol and some related drugs on the intraocular pressure (IOP) were studied after topical application in rabbits. These drugs produced a significant reduction in IOP with the following order of potency: (−)-propranolol > timolol > (±)-propranolol > sotalol > (+)-propranolol > pranolium. Pretreatment of rabbit eyes with atropine significantly antagonized the ocular hypotensive action of (±)-propranolol, timolol and pranolium. Both (±)-propranolol and timolol produced a significant increase in pupil diameter in the presence of a submydriatic dose of atropine. The activities of monoamine oxidase and carbonic anhydrase were unaffected by (±)-propranolol, timolol and pranolium in-vitro. It is concluded from the results that both cholinergic and adrenergic mechanisms may be involved in the ocular hypotensive effects of the drugs.

Both timolol and propranolol have been reported to reduce the intraocular pressure (IOP) in rabbits and in glaucoma patients (Radius et al 1978; Vale & Phillips 1970). However, the exact mechanism by which these drugs produce an ocular hypotensive action remains unclear (Chiou 1981). The cholinesterase enzyme inhibitory activities for propranolol and other related drugs have been reported by Alkondon et al (1983). In view of this, we have explored the possible involvement of a cholinergic mechanism in the ocular hypotensive effect of some β-blockers.

Materials and methods

Experiments on rabbits. Albino rabbits of either sex (1·5–2·0 kg), were housed under standard conditions and exposed to a 12 h light-dark cycle. Intraocular pressure was measured by means of a Schioetz tonometer (Medicon Instruments, Germany). A large number of preliminary IOP readings were made after local anaesthesia with 1% lignocaine in order to accommodate the animals to the measurement procedure. However, during the actual experiments, the readings were made after sedating the animals with diazepam (17·5 μmol kg^{-1} i.m.), 30 min before the start of IOP measurements. All drug studies were carried out in a blind fashion by two experimenters, one applying the drug (or vehicle) and the second, who had no knowledge of the schedule of drug administration until the end of the entire study, measuring the IOP. Six rabbits received (in two 50 μl doses, 5 min apart) drug dissolved in phosphate buffered saline, pH 7·4 on one eye and saline (vehicle) on the contralateral eye, into the inferior conjunctival sac. The six test drugs used were coded as D_1 to D_6 and each of the six animals received one of the drugs on day 1. On day 2, the drugs were given (by changing the order) to the eye which received vehicle on the first day. This procedure was repeated till the 6th day by which time, each animal had been exposed to all six drugs. These test drugs were administered in a concentration of 15 mM, since this approximately equals the concentrations of timolol and propranolol used in clinical and experimental situations (i.e. 0·5%).

The IOP measurements were made at 0, 30 60, 90, 120 and 180 min after drug or vehicle administration. In a second group of rabbits, the IOP readings were taken only after application of vehicle on both eyes. In another group, atropine was given 30 min before the test drugs and the IOP measurements observed. In all the animals, the corneal reflex was tested with a cotton swab, whereas the pupil diameter was measured by

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using a pupillometer before and after administration of drugs or vehicle.

Estimation of carbonic anhydrase activity. The enzyme activity was evaluated by the method of Wilbur & Anderson (1948) by using the rat kidney homogenates as the source of carbonic anhydrase enzyme.

Estimation of monoamine oxidase (MAO) activity. Partially purified rabbit serum was used as the source of MAO enzyme and its activity was determined by the method of McEwen (1971) with benzylamine as the substrate.

Drugs. (±)-, (+)-, (−)-Propranolol HCl (ICI), timolol maleate (Merck Sharp & Dohme), Sotalol HCl (Mead Johnson & Co) and pranolium iodide (*N,N*-dimethyl propranolol, UM-272 iodide, G. D. Searle & Co.), were used. Other agents were atropine salicylate (Boehringer Ingelheim), acetazolamide (Zolamide, Shalaks Pharmaceuticals Pvt Ltd), nialamide (Sigma) and diazepam (Ranbaxy Laboratories Ltd). Diazepam was dissolved in absolute alcohol and nialamide in a few drops of 1 M HCl and then in distilled water.

Statistical analysis. The results were analysed statistically by using 'paired' and 'unpaired' *t*-tests, where appropriate.

Results

Effect of drugs on rabbit eyes. Administration of phosphate saline (vehicle) into rabbit eyes did not produce any significant change in the IOP during the course of the experiment, i.e. up to 180 min. However, administration of propranolol, its isomers, timolol, sotalol and pranolium 0·1 ml, at a concentration of 15 mM, produced a significant reduction in the IOP of ipsilateral eyes (Fig. 1). This ocular hypotensive effect became evident within 30 min, reached a peak in 60 to 90 min and there was a partial recovery towards the initial pressure at the end of 180 min after administration of the drugs. When the overall reduction in IOP during the 180 min was calculated by the 'area under the curve' method, the drugs exhibited the following order of potency: (−)-propranolol > timolol > (±)-propranolol > sotalol > (+)-propranolol > pranolium (Table 1). (±)-Propranolol and timolol also caused reduction in IOP in the contralateral vehicle-treated eyes at 60 to 120 min and 60 to 90 min, respectively, whereas the other drugs had no influence on the IOP of contralateral eyes.

Reduction in IOP was associated with loss of the corneal reflex in ipsilateral eyes of rabbits treated with (±)-propranolol, (+)-propranolol or (−)-propranolol, which lasted for 50 ± 10, 48 ± 7·3 and 24 ± 11·2 min (n = 6 each), respectively. Applications of timolol, sotalol and pranolium did not cause any change in the corneal reflex. None of these agents at the concentration used produced any significant change in the diameter of the pupil.

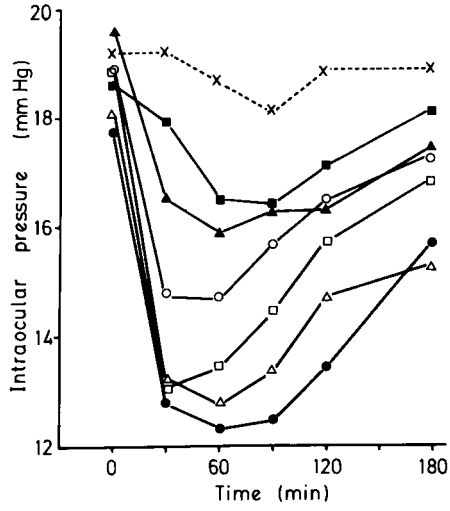

FIG. 1. Changes in intraocular pressure of rabbits produced by (×) vehicle, (■) pranolium, (▲) sotalol, (○) (+)-propranolol, (□) (±)-propranolol, (●) (−)-propranolol and (△) timolol. All agents were instilled into the inferior conjunctival sac in a volume of 0·1 ml and at a concentration of 15 mM for the drugs. Each point represents the mean data from six rabbits (standard error in all cases, less than 10% of mean values).

Table 1. Effect of propranolol and related drugs (with potency ratio) and their interaction with atropine, on the intraocular pressure (IOP) of rabbit eye.

Drugs	Reduction in IOP (AUC cm²) (Mean ± s.e.) (n = 6)	Potency ratio
(±)-Propranolol (P)	45·4 ± 4·21	1·000
(+)-Propranolol	34·0 ± 9·18	0·748
(−)-Propranolol	47·7 ± 7·40	1·050
Timolol	46·3 ± 7·05	1·019
Sotalol	34·1 ± 3·37	0·751
Pranolium	19·4 ± 6·18	0·427
Atropine	6·8 ± 1·20	—
Atropine + (±)-propranolol	10·0 ± 3·34***	—
Atropine + timolol	10·8 ± 4·47**	—
Atropine + pranolium	2·3 ± 2·33*	—

AUC = Area under the curve, calculated by plotting the observed reduction in mmHg against various time intervals studied up to 180 min, on a cm graph paper, for each animal after treatment.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Interaction with atropine. Administration of atropine (0·1 ml of 1·43 mM) itself produced a slight decrease in IOP (Table 1) and a slight increase in pupil diameter (Fig. 2), both changes being statistically insignificant. However, pretreatment of eyes with atropine in the

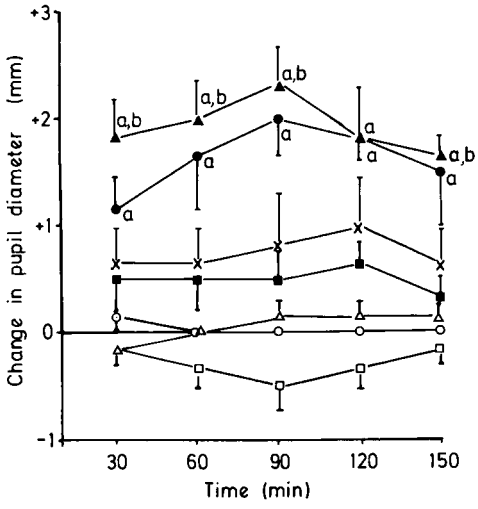

FIG. 2. Changes in pupil diameter of rabbits produced by (±)-propranolol (○), timolol (△), pranolium (□), atropine (×), atropine + (±)-propranolol (●), atropine + timolol (▲) and atropine + pranolium (■). Doses of the drugs, as in the text. Each point is the mean of data from six rabbits. Vertical bar represents standard error of the mean. (a) $P < 0.05$ (paired t-test); (b) $P < 0.05$ (unpaired t-test; data compared to atropine alone).

above dose 30 min previously, significantly antagonized the ocular hypotensive effects of (±)-propranolol, timolol and pranolium (Table 1). Interestingly, both (±)-propranolol and timolol produced a significant increase in pupil diameter only in the presence of atropine (Fig. 2).

Effect of drugs on enzyme activity. Acetazolamide (1×10^{-8} to 1×10^{-6} M) produced a dose-dependent inhibition (17 to 100%) of carbonic anhydrase activity of rat kidney homogenates. However, (±)-propranolol (up to 1×10^{-2} M), timolol (up to $2{\cdot}5 \times 10^{-3}$ M) and pranolium (up to 1×10^{-3} M) failed to produce any significant inhibition of the enzyme activity. Similarly, the monoamine oxidase enzyme activity of purified rabbit serum was inhibited (1·5 to 98·3%) only by nialamide (1×10^{-6} to 10^{-4} M) but not by (±)-propranolol, timolol and pranolium up to 1×10^{-3} M.

Discussion

Wide angle glaucoma is a pathological condition where there is elevated IOP which can be alleviated by drug therapy (Taylor 1980). A wide variety of drugs, like cholinergic agents (pilocarpine, physostigmine), adrenergic agents (adrenaline, isoprenaline), carbonic anhydrase inhibitors (acetazolamide), MAO inhibitor (pargyline) and β-adrenergic blocking agents (timolol), have been found to be effective in reducing the IOP in glaucoma patients (Mehra et al 1974; Chiou 1981). Though it has been suggested that timolol may exert its ocular hypotensive effect through reduction in aqueous humour formation (Boger et al 1978), the actual mechanism of action remains unclear (Chiou 1981). Both propranolol and timolol have been found to be effective in reducing the IOP in man as well as in rabbits (Vale & Phillips 1970; Radius et al 1978). By using propranolol and related drugs with differing β-receptor blocking potencies and other ancillary effects, it was possible in the present study to delineate the relationship between the ocular effects and their various properties. The present results clearly indicate a significant ocular hypotensive effect for propranolol, its isomers, timolol, sotalol and the dimethyl quaternary analogue of propranolol pranolium.

The presence of β-adrenoceptors in the ciliary processes has been demonstrated (Nathanson 1980) and it has also been postulated that the β-adrenoceptor antagonists like timolol and IPS 339 ((t-butylamino-3-ol-2-propyl)oximino-9-fluorene HCl) may decrease the secretion of aqueous humour and thereby decrease the IOP by acting on these receptors (Nathanson 1981). On the contrary, the β-adrenoceptor stimulants like salbutamol and metaproterenol have been reported to reduce the IOP in rabbits (Potter & Rowland 1978). The observed order of potency for the reduction in IOP by propranolol and other drugs used also suggests an involvement of β-adrenoceptor blockade in the above action since the potent β-adrenoceptor antagonists like timolol, (−)-propranolol and (±)-propranolol (Barrett & Cullum 1968; Scriabine et al 1973) were found to be more effective on a molar basis than the other agents like sotalol and (+)-propranolol (both being weak β-adrenoceptor antagonists, Barrett & Cullum 1968) and pranolium (which has no β-blocking property, Schuster et al 1973). However, a strict correlation between the two phenomena was not observed since the reported ratio of potency among timolol, (−)-propranolol and (±)-propranolol for β-adrenoceptor blockade was 6:2:1, whereas we found that the ratio for reduction of IOP in rabbits was 1·019:1·050:1·000. Moreover, the propranolol analogue, pranolium, which has no β-adrenoceptor activity (Schuster et al 1973) also produced a reduction in IOP in rabbits thereby suggesting the involvement of other mechanisms.

Apart from blocking β-adrenoceptors, propranolol has been reported to possess other properties, e.g. a local anaesthetic action (Barrett & Cullum 1968) and a cholinesterase enzyme inhibitory effect (Alkondon et al 1983). Since the concentration of propranolol, timolol and other drugs applied to the eye to produce an ocular hypotensive action is in the order of mM range, it is possible that these drugs might reduce the IOP. That atropine significantly antagonized the ocular hypotensive action of (±)-propranolol, timolol and pranolium suggests the involvement of a cholinergic mechanism. The loss of the corneal reflex observed in rabbits after local application of propranolol and its isomers, corroborates with the local anaesthetic properties for these compounds (Barrett & Cullum 1968). However, there

appears to be no relationship between this property and the ocular hypotensive effect since timolol, sotalol and pranolium exhibited a reduction in IOP in the absence of a local anaesthetic activity. Moreover, the decrease in the IOP outlasted the duration of local anaesthesia for propranolol and its isomers.

Both racemic propranolol and timolol produced a significant increase in pupil diameter only in the presence of atropine. Since atropine is known to inhibit the cholinergic drive to the iris muscles, the observed mydriatic effect of (±)-propranolol and timolol in the presence of atropine may point towards the involvement of an increased adrenergic tone in the dilator pupillae, which otherwise might have been masked by an opposing cholinergic influence. Such an increased adrenergic activity seems conceivable in view of the observation that propranolol is able to prevent the reuptake of noradrenaline in cardiac tissue (Foo et al 1968). Since both cholinergic and adrenergic agonists are known to reduce the IOP (Chiou 1981), the activation of both the systems by (±)-propranolol and timolol may lead to a greater reduction of ocular pressure. This may also explain an absence of a significant change in pupil diameter with these agents. A similar hypothesis (i.e. the involvement of both adrenergic and cholinergic mechanisms) has also been suggested by Colasanti & Trotter (1979), who observed a reduced ocular hypotensive action of timolol in cats after sympathectomy or after rendering the cat eyes subsensitive to the pressure-lowering effects of cholinomimetics by chronic echothiophate treatment.

The enzymes carbonic anhydrase and monoamine oxidase play a significant modulatory role in the regulation of intraocular pressure, and drugs which inhibit these enzymes are effective ocular hypotensive agents (Chiou 1981; Mehra et al 1974). The failure of (±)-propranolol, timolol and pranolium to inhibit carbonic anhydrase and monoamine oxidase enzyme activity in the present study indicates that their mechanism in reducing the IOP is not similar to that of acetazolamide (carbonic anhydrase inhibitor) and pargyline (monoamine oxidase inhibitor), both of which are reported to reduce the IOP in glaucoma patients.

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