Improved Delivery through Biological Membranes. 26. Design, Synthesis, and Pharmacological Activity of a Novel Chemical Delivery System for β-Adrenergic Blocking Agents

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Novel ketoxime analogues of known β-blockers (propranolol, timolol, carteolol) were synthesized and tested as potential site-specific chemical delivery systems. It was assumed that a hydrolysis–reduction sequence could produce the active β-blockers in the iris–ciliary body. It was found that some of these bioprecursors are remarkably active in reducing intraocular pressure in rabbits. The ketoxime derivative of propranolol is more effective and much less irritant than its parent β-blocker. While the ketoximes also displayed activity on isoprenaline-induced tachycardia after iv administration, they were void of activity when given orally. Propranolol was found for a prolonged time and in significant concentrations in the rabbit's eye following topical administration of its ketoxime precursor; however, the inactive ketoximes apparently were not converted to the corresponding β-blockers in the eye. A correlation was found between the physicochemical properties of the ketoximes and their conversion to the amino alcohol and thus their subsequent activity. The results suggest that at least some of the ketoxime precursors could have a use as antiglaucoma agents without systemic side effects.

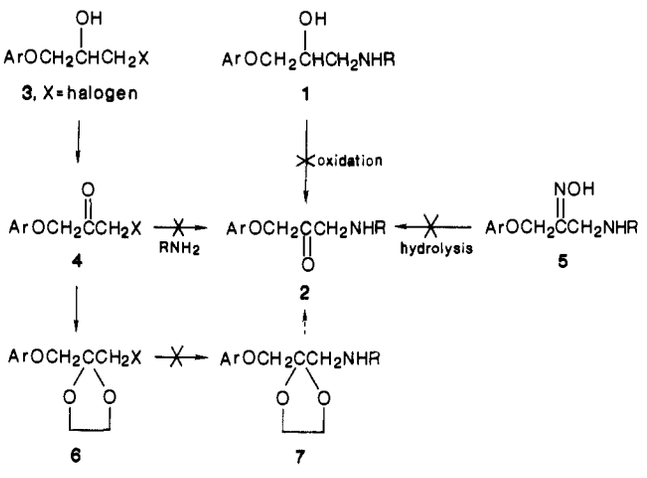
β-Adrenergic blockers were first reported to be useful for the drug treatment of glaucoma in 1967.[3] In 1978 timolol was approved for market use, and since that time, it has become very popular with ophthalmologists as an effective antiglaucoma agent. Recently, however, a vast number of serious cardiovascular, respiratory, central nervous system (CNS), and ocular side effects secondary to topical ocular timolol administration have been reported.[4-9] Nowadays, timolol is no longer the sole β-blocker used to treat glaucoma. Befanolol and carteolol are marketed now in Japan, metipranolol was introduced recently into the German market, and a number of other newer β-adrenergic antagonists (e.g., L-bunolol, betaxolol, celiprolol, cetamolol, etc.) are currently under investigation as antiglaucoma agents.

It would be most desirable to design an antiglaucoma drug that could be delivered to the eye compartments in a sustained and controlled manner with the least possible systemic absorption and/or no systemic side effects.

We have previously found that, after topical application to the eye, esters of adrenalone but not adrenalone itself can be converted via a reduction–hydrolysis sequence to deliver adrenaline (epinephrine) *only* at the iris–ciliary body, the site of action.[10] This suggested that lipophilic ketones can be reduced in the iris–ciliary body.

Accordingly, ketone precursors of β-blockers that are also β-hydroxylamines like adrenaline could then possibly be converted to the active β-blockers in the iris–ciliary body by a reductive process. Various attempts, however, to synthesize the ketones corresponding to a number of β-blockers (propranolol, timolol, carteolol, etc.) have failed, due to the chemical instability of these β-amino ketone ethers. In order to stabilize the ketone precursors, the hydrolytically sensitive oxime function was considered. Thus, a hydrolysis–reduction sequence could produce the active amino alcohol at the iris–ciliary body, the site of action, from the ketoxime type chemical delivery system (CDS).

Scheme I



Here we report studies on the design, synthesis, and pharmacological activity of a novel CDS for certain β-adrenergic antagonists, based on the above concept, and in particular the potential use of the ketoxime precursors of these drugs in the treatment of glaucoma.

Results and Discussion

Chemistry. The initial target compounds were the amino ketones corresponding to the β-blockers propranolol (**1a**), timolol (**1b**), carteolol (**1c**), and the *N*-isopropyl analogue of timolol (**1d**). The attempted synthetic routes leading to the derived ketones are summarized in Scheme I. Oxidation of **1a**, **1b**, or **1c** under various conditions used for conversion of 1,2-amino alcohols to ketones ($Na_2Cr_2O_7$ with sulfuric acid) or acetic acid and $(t\text{-BuO})_3Al$, benzophenone,[11,12] DCC–DMSO in various conditions,[13] etc., has

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(3) Phillips, C. I.; Howitt, G.; Rowlands, D. J. *Br. J. Ophthalmol.* **1967**, *51*, 222.
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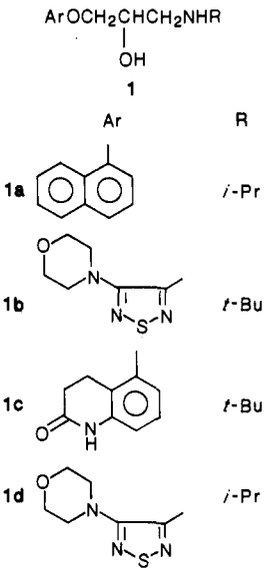
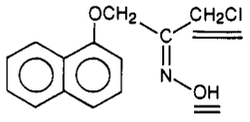
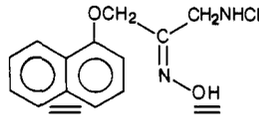


failed to produce the ketones. While **4** (X = Cl) can easily be made by oxidation of **3**, its reaction with RNH_2 has always yielded ArOH. Conversion of **4** to the ketal **6** could be accomplished, but the amino ketal **7** was not obtained when **6** was reacted with RNH_2. It was thus decided to use a stable, but hydrolytically sensitive, ketone derivative as the potential chemical delivery system for **1**. The oximes **5a–d** were first selected as target compounds. Their synthesis is shown in Scheme II.

The conventional reaction of ArOH and epichlorohydrin with a small amount of morpholine as catalyst afforded a mixture of the chlorohydrin **8** and the epoxide **9**; the latter was converted to **8** by treatment with concentrated HCl. Oxidation of **8** by the Pfitzner–Moffat method[13] yielded the ketone **10**. Subsequent reaction of **10** with hydroxylamine hydrochloride gave the oxime **11**, which is a mixture of the *Z* and *E* isomers. In the case of **11a**, the



11a(*Z*)



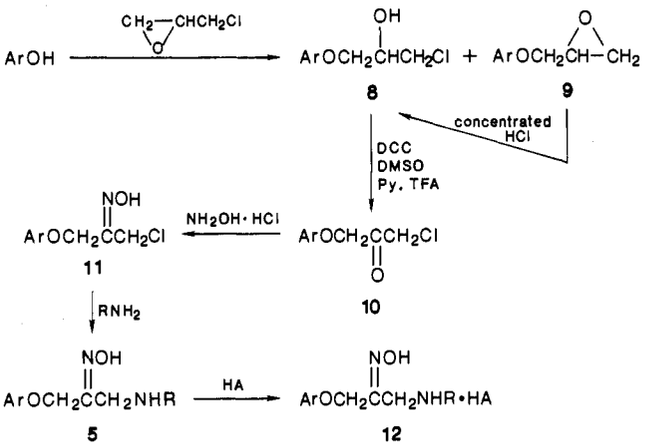
12a(*E*)

ratio was about 2:1, as determined by NMR.[14] The separation of the two methylene signals is greater in the *Z* isomer in **11a** than in the *E* isomer. The major product, **11a**(*Z*), could be isolated by recrystallization from benzene. Treatment of **11a**(*Z*) with isopropylamine in THF gave propranolone oxime (**5a**) essentially as the pure *E* isomer, which was converted to the HCl salt **12a**.

The other oximes, **12b–d**, were synthesized and purified in a manner similar to that used for **12a**. All final oximes were pure *E* isomers.

Pharmacology. The pharmacological properties of the newly synthesized ketoximes were evaluated in comparison with those of their respective known parent β-adrenergic antagonists. At first, the effect of the different compounds on the intraocular pressure (IOP) of normotensive rabbits was assessed. Results revealed that the ketoxime analogues of both propranolol and timolol display a certain degree of ocular hypotensive activity (Figure 1). Propranolone oxime (**12a**) has shown the highest activity at both tested concentration levels, 1% and 2.5%. This activity was much more pronounced and prolonged than that of pro-

Scheme II



pranolol itself administered at the same dose levels (Tables I and II). In addition, the ketoxime **12a** was completely devoid of the ocular irritation that always accompanied propranolol administration at both dose levels. This irritant activity might have contributed to the reduced action of propranolol on the IOP at the 1% dose level and, also, might have completely masked its ocular hypotensive activity at the 2.5% dose level. Timolone oxime (**12b**) has also shown a significant ocular hypotensive activity, which was faster in its onset and shorter in its duration than that of timolol (**1b**) itself (Table III). On the other hand, the other ketoxime precursors, the ones for the *N*-isopropyl analogue **12d** of **12b** and **12c** for carteolol, were almost completely devoid of any action on the IOP of rabbits.

In the second set of pharmacologic experiments, the effect of the different compounds on the resting heart rate of rats was evaluated following iv administration. Results of this study revealed that most of the tested ketoximes exhibit a negative chronotropic action in rats (Figure 2). Again, the ketoximes of propranolol (**12a**) and timolol (**12b**) have shown the highest activity in this test, whereas carteolone oxime (**12c**) and the oxime **12d** were less active. It should be mentioned also that in this test carteolol (**1c**) itself has shown the lowest activity on the heart rate of rats.

The potential β-adrenergic antagonistic activity of the ketoxime precursors of propranolol, timolol, and carteolol was assessed against isoprenaline tachycardia by using the parent compounds as obvious reference drugs. Results of this set of experiments were in agreement with the findings of the above-mentioned two studies, effect on the IOP and resting heart rate. Thus, the ketoxime precursors of propranolol (**12a**) and timolol (**12b**) were the most effective whereas **12c** and **12d** were the least active (Figure 3).

These results indicate that at least two of the investigated ketoxime precursors (**12a** and **12b**) have a potential antiglaucoma activity which could probably be linked to their β-adrenergic antagonistic properties. Yet, whether these properties are due to an inherent intrinsic activity of the ketoximes themselves or are the result of their active biological conversion to their parent drugs needed to be verified. For this reason, the in vivo disposition of the different ketoximes and their parent β-blockers in the different ocular tissues was studied in rabbits. Results of this study have shown that propranolol (**1a**) could be detected in measurable concentrations in the different eye compartments for the first 2 h following the topical administration of its ketoxime precursor **12a** at its effective ocular hypotensive dose level (1%) (Table IV). On the other hand, propranolol could not be detected in any of the tested eye tissues 2 h after its ocular application (Table IV), and it had completely disappeared from the iris–ciliary

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Table I. Effect of 1% Solutions of Propranolol Hydrochloride (**1a**) and Propranolone Oxime Hydrochloride (**12a**) on the IOP (mmHg) of Rabbits[a]

time after administration	propranolol hydrochloride (**1a**), 1%			propranolone oxime hydrochloride (**12a**), 1%		
	control	treated	% change after treatment	control	treated	% change after treatment
0 min	28.8 ± 0.33	30.8 ± 0.40	0.00	29.3 ± 0.50	27.8 ± 0.62	0.00
30 min	32.0 ± 0.44	2,90 ± 0.56	-9.10*	28.2 ± 0.65	26.9 ± 0.58	-3.23
60 min	32.7 ± 0.26	27.6 ± 0.62	-10.39*	29.1 ± 0.60	24.0 ± 0.60	-11.51*
2 h	31.3 ± 0.31	29.1 ± 0.38	-5.52	27.7 ± 0.57	23.4 ± 0.51	-15.82**
3 h	30.8 ± 0.32	27.4 ± 0.54	-11.04*	26.3 ± 0.40	23.3 ± 0.35	-16.18**
4 h	29.9 ± 0.61	28.2 ± 0.48	-8.44*	26.8 ± 0.33	22.2 ± 0.42	-20.14**
6 h	30.8 ± 0.38	2.90 ± 0.45	-5.84	28.8 ± 0.52	25.8 ± 0.56	-7.08*
8 h	30.7 ± 0.26	30.2 ± 0.43	-1.95	29.2 ± 0.51	27.9 ± 0.62	0.50

[a] *Significant decrease in IOP ($P > 0.05$). **Highly significant decrease in IOP ($P > 0.01$).

Table II. Effect of 2.5% Solutions of Propranolol Hydrochloride (**1a**) and Propranolone Oxime Hydrochloride (**12a**) on the IOP (mmHg) of Rabbits[a]

time after administration	propranolol hydrochloride (**1a**), 2.5%			propranolone oxime hydrochloride (**12a**), 2.5%		
	control	treated	% change after treatment	control	treated	% change after treatment
0 min	26.6 ± 0.56	25.4 ± 0.56	0.00	25.8 ± 0.55	26.0 ± 0.48	0.00
30 min	28.0 ± 0.93	27.6 ± 0.5l	8.66*	27.2 ± 0.82	26.5 ± 0.63	1.92
60 min	26.6 ± 0.51	27.9 ± 0.60	9.84*	26.2 ± 0.63	23.4 ± 0.55	-10.00*
2 h	24.6 ± 0.19	25.7 ± 0.55	1.18	26.3 ± 0.71	22.6 ± 0.43	-13.08*
3 h	25.6 ± 0.31	25.7 ± 0.39	1.18	26.7 ± 0.66	21.2 ± 0.28	-18.46*
4 h	25.2 ± 0.64	25.6 ± 0.46	0.79	26.8 ± 0.34	20.4 ± 0.34	-21.54**
6 h	26.4 ± 0.35	25.4 ± 0.54	0.00	25.9 ± 0.54	23.6 ± 0.48	-9.23*
8 h	26.0 ± 0.52	25.2 ± 0.68	-0.79	26.0 ± 0.47	25.8 ± 0.65	-0.77

[a] *Significant change ($P > 0.05$). **Highly significant change ($P > 0.01$).

Table III. Effect of 1% Solutions of Timolol Maleate (**1b**) and Timolone Oxime Oxalate (**12b**) on the IOP (mmHg) of Rabbits[a]

time after administration	timolol maleate (**1b**), 1%			timolone oxime oxalate (**12b**), 1%		
	control	drug treated	% change after treatment	control	drug treated	% change after treatment
0 min	28.72 ± 0.54	29.19 ± 0.78	0.00	28.72 ± 0.54	28.44 ± 0.63	0.00
30 min	26.62 ± 0.59	26.00 ± 1.23	-10.93*	26.62 ± 0.59	27.75 ± 0.68	-2.43
1 h	28.10 ± 0.85	27.25 ± 1.12	6.65	28.10 ± 0.85	24.30 ± 0.63	-14.56**
2 h	27.02 ± 0.92	27.21 ± 0.58	6.78	27.02 ± 0.92	24.00 ± 0.44	-15.61**
3 h	25.95 ± 0.44	25.00 ± 0.87	14.35**	25.95 ± 0.44	25.13 ± 0.96	-11.64**
4 h	26.45 ± 0.57	24.88 ± 1.00	14.77**	26.45 ± 0.57	26.93 ± 0.48	-5.31
5 h	27.09 ± 0.55	24.25 ± 1.18	16.92**	27.09 ± 0.55	26.88 ± 0.52	-5.49
6 h	27.88 ± 0.56	25.58 ± 0.85	12.37**	27.88 ± 0.56	28.78 ± 0.91	1.20
8 h	27.67 ± 0.63	26.33 ± 0.40	9.80*	27.67 ± 0.63	26.33 ± 0.40	-7.42

[a] *Significant change ($P > 0.05$). **Highly significant change ($P > 0.01$).

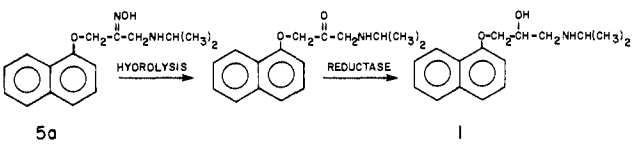
Table IV. Tissue Concentration[a] of Propranolol (**1a**) and Propranolone Oxime (**5a**) at Various Time Intervals following Topical Administration of Propranolol Hydrochloride (**1a**) and Propranolone Oxime Hydrochloride (**12a**) (1% Solutions)

compd	time, min	concn of propranolol (**1a**) ± SE, μg/g			concn of propranolone oxime (**5a**) ± SE, μg/g		
		cornea	iris-ciliary body	aqueous humor	cornea	iris-ciliary body	aqueous humor
1a	30	47.1 ± 5.6	8.1 ± 1.5	1.3 ± 0.2	N/A[c]	N/A	N/A
	60	14.5 ± 3.0	*b*	0.3 ± 0.1	N/A	N/A	N/A
	120	*b*	*b*	*b*	N/A	N/A	N/A
12a	30	1.7 ± 0.8	2.1 ± 0.3	*b*	23.8 ± 4.9	7.8 ± 1.1	0.8 ± 0.1
	60	1.1 ± 0.3	1.8 ± 0.2	0.7 ± 0.1	16.4 ± 5.8	*b*	0.8 ± 0.1
	120	1.1 ± 0.2	0.4 ± 0.1	*b*	*b*	*b*	*b*

[a] Figures represent the mean ± SE of the mean of at least four rabbits. [b] Below detection limit. [c] N/A: no oxime can be present after administration of propranolone hydrochloride.

body, which is supposed to be the site of its ocular hypotensive action, 1 h after administration. These results might explain the shorter duration of propranolol action on the IOP relative to that of its ketoxime precursor. In addition, these results might also suggest that the ocular hypotensive activity of the oxime is most probably due to its active conversion to propranolol in situ in the ocular tissues of rabbits (Scheme III). This is also supported by the finding that following the ophthalmic administration of the other ketoxime precursors (**12b** and **12c**) of timolol and carteolol, respectively, we were not able to detect the parent β-adrenergic antagonists in any of the eye compartments. This would suggest that either the ketone

Scheme III



5a I

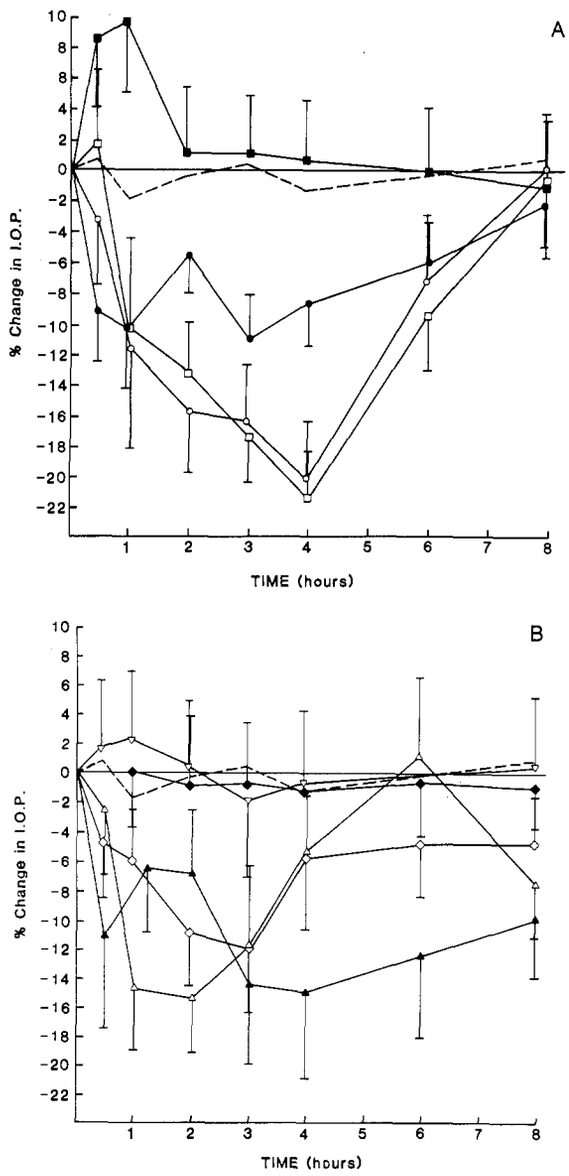


Figure 1. A. Effect of propranolol hydrochloride (**1a**) and propranolone oxime hydrochloride (**12a**) on the intraocular pressure of rabbits; (●) propranolol hydrochloride (**1a**) (1%), (■) propranolol hydrochloride (**1a**) (2.5%), (○) propranolone oxime hydrochloride (**12a**) (1%), (□) propranolone oxime hydrochloride (**12a**) (2.5%), and (- -) saline solution (n = 6–10). B. Effect of the different β-blockers and their ketoxime precursors on the IOP of rabbits; (▲) timolol maleate (**1b**) (1%), (◇) carteolol hydrochloride (**1c**) (1%), (△) timolone oxime oxalate hydrochloride (**12b**) (1%), (▽) N-isopropyl analogue of timolone oxime hydrochloride (**12d**) (1%), (◆) carteolone oxime hydrochloride (**12c**) (1%), and (- -) saline solution (n = 6–10).

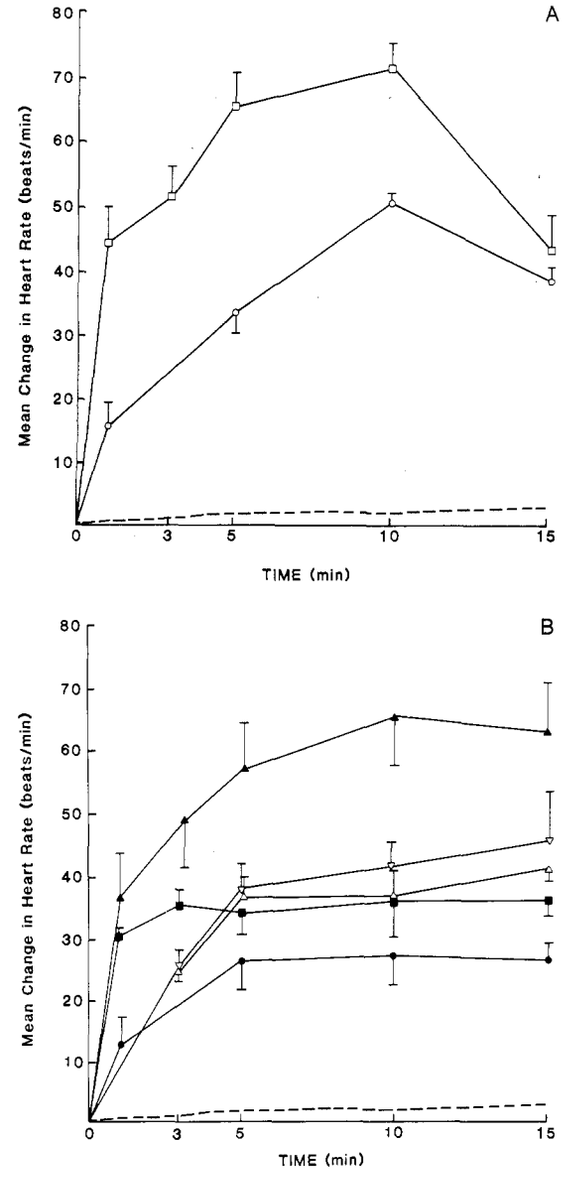


Figure 2. A. Effect of the iv injection of 6 mg/kg of propranolol hydrochloride (**1a**) (□), propranolone oxime hydrochloride (**12a**) (○), and saline solution (- -) on the resting heart rate of rats (n = 7). B. Effect of the iv injection of the different β-blockers and their ketoxime precursors (6 mg/kg) on the resting heart rate of rats; (▲) timolol maleate (**1b**), (●) carteolol hydrochloride (**1c**), (▽) timolone oxime oxalate (**12b**), (△) N-isopropyl analogue of timolone oxime hydrochloride (**12d**), (■) carteolone oxime hydrochloride (**12c**), and (- -) saline solution (n = 7).

formed or the reduced form, the active β-blocker, is disposed of so fast that it cannot be detected or, more likely, that the ketones (**2b** and **2c**) are not good substrates for the reductase enzyme. On the basis of the previous observation[10] on the necessity to convert adrenalone to lipophilic esters to be reduced, one could expect that the lipophilic propranolone is easily reduced, while the ketones derived from timolol and carteolol, being less lipophilic (heterocyclic substitution of naphthalene), are not. The N-isopropyl analogue **12d** of timolone oxime was synthesized and tested in order to assess the importance of the N-alkyl function. Propranolone oxime contains an isopropyl group, like **12d**, but **12d** was still found inactive. The difference in the behavior of **12a** vs **12b–d** thus might be due to the difference in the Ar group, which appears to determine the substrate properties necessary to bind to the reductase enzyme. This hypothesis is supported by the relative HPLC retention times of the free bases **5a–d**, which were 12.86, 7.22, 3.10, and 6.10 for **5a**, **5b**, **5c**, and **5d**, respectively, indicating that **5a** is by far the most lipophilic.

It was also necessary to follow up the biotransformation pathway of propranolone oxime (**12a**) following its systemic administration. These studies revealed that the metabolic pathway of the oxime in the blood is quite different from that in ocular tissues. Thus, propranolol was not detected in rat blood following the iv administration of the oxime, and instead, another, more polar compound was detected. However, 5 min after injection, even this compound had totally disappeared. The oxime itself appeared to have a very fast metabolism in blood (Figure 4). The $t_{1/2}$ in blood was equivalent to 7.64 ± 0.55 min, and 1 h after iv ad-

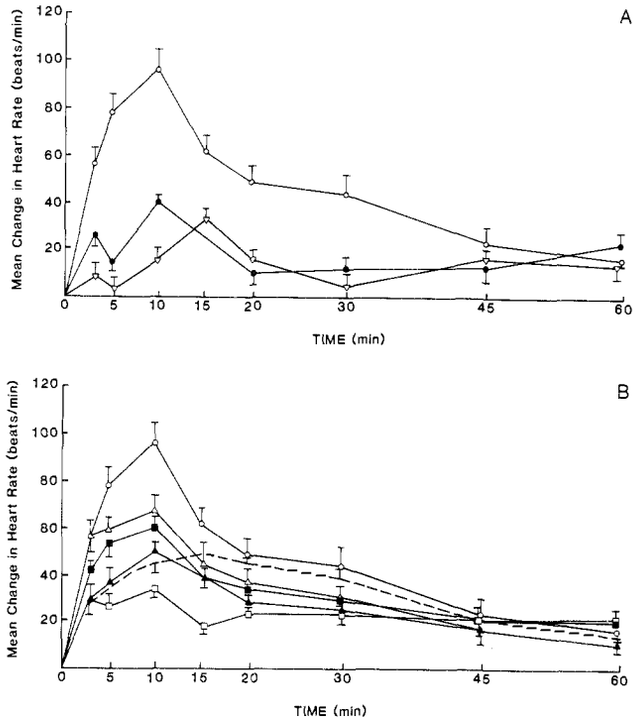


Figure 3. A. Mean change in heart rate following administration of isoproterenol (50 μg/kg, sc). Each group was pretreated with either (▽) propranolol hydrochloride (**1a**), (●) propranolone oxime hydrochloride (**12a**), or (○) saline solution. Each compound was administered intravenously at a dose of 6 mg/kg 15 min prior to administration of isoproterenol (n = 7). B. Mean change in heart rate following administration of isoproterenol (50 μg/kg, sc). Each group was pretreated with either (– –) timolol maleate (**1b**), (▲) carteolol hydrochloride (**1c**), (□) timolone oxime oxalate (**12b**), (■) N-isopropyl analogue of timolone oxime hydrochloride (**12d**), (△) carteolone oxime hydrochloride (**12c**), or (○) saline solution. Each compound was administered intravenously at a dose of 6 mg/kg 15 min prior to administration of isoproterenol (n = 7).

ministration, the oxime had completely disappeared from the blood.

While these results would suggest that the propranolol formed in situ in the iris–ciliary body is responsible for the IOP reduction observed, the ketoxime itself might have intrinsic activity. Further studies to clarify the extent of β-blocking activity of **12a** and its possible metabolites are currently under way.

Although it is most likely that the corresponding β-blockers are produced also systemically when injected iv, oral administration (which is the way drugs topically applied to the eye reach the system) of **12a** at 25–100 mg/kg dose levels did not show β-adrenergic antagonist activity.

Regardless of the intimate mechanism of action (bioactivation or intrinsic activity), the ketoxime **12a** represents a novel, nonirritating, and effective IOP reducing agent.

Experimental Section

Chemistry. Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Elemental analysis was performed by Atlantic Microlab, Inc., Atlanta, GA. The 90-MHz NMR spectra were taken on a Varian EM390 NMR spectrometer. TLC was performed on 0.25-mm Merck silica gel 60 F-254 glass plates.

The synthesis of propranolone oxime (**12a**) is a typical example for general Scheme II.

3-Chloro-1-(1-naphthyloxy)-2-propanol (8a). A mixture of 1-naphthol (20 g, 0.14 mol), epichlorohydrin (51.3 g, 0.55 mol), and morpholine (0.7 mL) was heated at 100–120 °C for 7.5 h. Excess epichlorohydrin and morpholine were removed under reduced pressure, and the residue was dissolved in chloroform

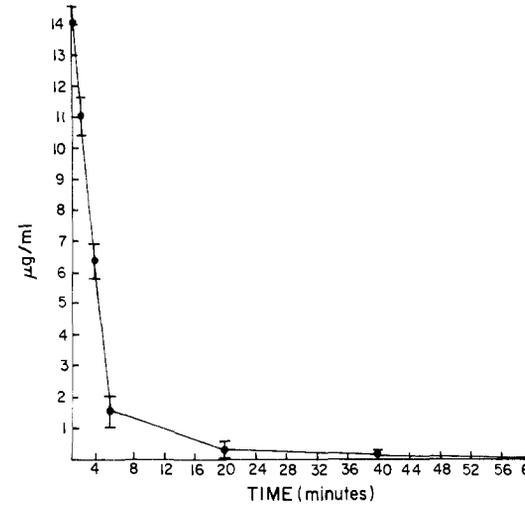


Figure 4. Blood levels vs time of propranolone oxime (**5a**) following iv administration of propranolone oxime hydrochloride (**12a**) at a dose level of 6 mg/kg to rats (n = 7).

and shaken with 10 mL of concentrated HCl to convert **9a** to the chlorohydrin **8a**. The organic layer was separated and washed with water, then with dilute $NaHCO_3$, and finally with water. It was dried over anhydrous $MgSO_4$ and concentrated to yield 29.8 g (98%) of the crude product. This was used in the next step (oxidation) without purification.

Purification of a sample of the crude **8a** was carried out by column chromatography (silica gel: Aldrich 100–200 mesh, 60 Å × 4W, eluent $CHCl_3$): NMR ($CDCl_3$) δ 8.15 (m, 1 H), 7.75 (m, 1 H) 7.5–7.2 (m, 4 H), 6.7 (d, d, J = 7 Hz, J = 1 Hz, 1 H), 4.35–3.50 (m, 5 H), 2.9 (d, J = 6 Hz, 1 H).

3-Chloro-1-(1-naphthyloxy)-2-propanone (10a). To a solution of 1,3-dicyclohexylcarbodiimide (DCC) (47.1 g, 0.228 mol), DMSO (36 mL), and pyridine (3.6 mL) in diethyl ether (300 mL) was added a solution of **8a** (18.0 g, 76 mmol) in diethyl ether (36 mL). To this solution was then added dropwise a solution of trifluoroacetic acid (1.8 mL) in diethyl ether under ice–water cooling, and the mixture was stirred at room temperature for 1 h and allowed to stand overnight. A solution of oxalic acid (18 g) in MeOH was added to the reaction mixture in small portions, and the stirring was continued for 0.5 h. The dicyclohexylurea was filtered and washed with ether. The filtrate was washed with a 5% $NaHCO_3$ solution and then with water and dried over anhydrous $MgSO_4$. From the filtrate was recovered 6.3 g of the desired compound. The mother liquor was concentrated under reduced pressure, and the residue was recrystallized from 2-propanol to yield and additional 3.3 g. The total yield was 9.6 g (56%). This product was used in the next step without further purification. A pure sample was obtained by column chromatography (silica gel: Aldrich 100–200 mesh, 60 Å × 7W, eluent $CHCl_3$–hexane, 3:1): NMR ($CDCl_3$) δ 8.25 (m, 1 H), 7.80 (m, 1 H), 7.65–7.20 (m, 4 H), 6.75 (d, J = 7 Hz, 1 H), 4.83 (s, 2 H), 4.43 (s, 2 H).

3-Chloro-1-(1-naphthyloxy)-2-propanone Oxime (11a). A mixture of **10a** (1.0 g, 4.26 mmol), hydroxylamine hydrochloride (0.36 g, 5.1 mmol), and DMSO (10 mL) was heated at 40–60 °C for a half-hour. Water (40 mL) was introduced, and the solution was extracted with $CHCl_3$. The organic layer was washed with water several times, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude yield was 1.1 g (100%). Further purification was carried out by column chromatography (silica gel: Aldrich 100–200 mesh, 60 Å × 30W, eleunt benzene–AcOEt, 4:1). The product was a mixture of Z and E isomers (Z:E = 2:1). This isomer mixture could be used in the next step: NMR ($CDCl_3$ + DMSO-d_6 (1 drop)) δ 10.85 (s, 0.33 H, NOH of E isomer), 10.75 (s, 0.67 H, NOH of Z isomer), 8.4–8.1 (m, 1 H), 7.9–7.65 (m, 1 H), 7.6–7.2 (m, 4 H), 6.95–6.7 (m, 1 H), 5.2 (s, 1.33 H, OCH_2 of Z isomer), 4.9 (s, 0.67 H, OCH_2 of E isomer), 4.45 (s, 0.67 H, CH_2Cl of E_1 isomer), 4.3 (s, 1.33 H, CH_2Cl of Z isomer).

The Z isomer was isolated from the crude product by recrystallization from benzene, mp 162–163 °C.

1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanone Oxime (Propranolone Oxime, 5a). A mixture of **11a** (2.5 g, 10 mmol), isopropylamine (6.0 g, 8.7 ml, 100 mmol), and THF (50 mL) was heated at 50 °C for 1.5 h. The reaction mixture was concentrated under reduced pressure. To the residue was added diluted $NaHCO_3$, and the solution was extracted with ethyl acetate. After the organic extract was shaken with dilute HCl solution, the separated aqueous layer was made basic with dilute $NaHCO_3$ solution, extracted with AcOEt, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The crude yield was 2.6 g (95%). The crude product was purified from a mixed solvent of isopropyl ether and hexane. The pure yield was 0.98 g (36%), mp 131.5–132.5 °C. This product was the *E* isomer only: NMR ($CDCl_3$) δ 8.30–8.20 (m, 1 H, one of H of naphthalene), 6.90–6.80 (m, 1 H, one of H of naphthalene), 5.16 (s, 2 H, OCH_2), 3.70 (s, 2 H, CH_2N), 3.05–2.70 (m, 1 H, NCH), 1.10.

1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanone Oxime Hydrochloride (Propranolone Oxime Hydrochloride, 12a). To diethyl ether saturated with HCl gas was added a solution of propranolone oxime (**5a**) (0.30 g) in diethyl ether. The mixture was stirred at room temperature for 0.5 h. The precipitated white crystals were filtered and dried in vacuo overnight. The yield was 0.32 g (94%). This product was essentially pure *E* isomer: NMR (DMSO-d_6) δ 12.00 (s, 1 H, NOH), 8.30–8.15 (m, 1 H, of naphthalene), 7.95–7.80 (m, 1 H, of naphthalene), 7.65–7.30 (m, 4 H, part of naphthalene), 7.05–6.95 (m, 1 H, one of H of naphthalene), 5.15 (s, 2 H, OCH_2), 3.96 (s, 2 H, CH_2N), 3.55–3.20 (m, 2 H, NCH, NH), 1.27 (d, J = 6 Hz, 6 H, $(CH_3)_2$). Anal. ($C_{16}H_{20}O_2N_2$·HCl) C, H, N.

3-Chloro-1-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol (8b) was synthesized according to the method given for **8a**: yield 83%; the crude compound was used in the next step; NMR ($CDCl_3$) δ 4.5 (d, J = 5 Hz, 2 H), 4.2 (pentet, J = 5 Hz, 1 H), 3.8–3.65 (m, 6 H), 3.55–3.40 (m, 4 H). The peak of COH cannot be identified.

3-Chloro-1-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanone (10b) was synthesized according to the method given for **10a**, yield 65%. The crude product was used in the next step. Purification of the crude product (recrystallization from 2-propanol) yielded the pure sample: NMR ($CDCl_3$) δ 5.22 (s, 2 H), 4.13 (s, 2 H), 3.75 (m, 4 H), 3.50 (m, 4 H).

3-Chloro-1-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanone Oxime (11b). In a 500-mL round-bottomed flask were placed **10b** (13.6 g, 49 mmol) and hydroxylamine hydrochloride (5.1 g, 73.4 mmol), in an ethanol–DMF mixed solvent (266 mL). The mixture was stirred at room temperature for 18 h. The reaction mixture was poured into water (2 L) and was extracted with ether. The organic extract was washed well with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo at 30 °C. The crude yield was 11.8 g (83%). The product was a mixture of *Z* and *E* isomers (*Z*:*E* = 1.1:1.0). This crude mixture can be used in the next step: NMR ($CDCl_3$) δ 9.30 (br s, 0.5 H, NOH of *E* isomer), 9.10 (br s, 0.5 H, NOH of *Z* isomer), 5.35 (s, 1 H, OCH_2 of *Z* isomer), 5.10 (s, 1 H, *E* isomer), 4.30 (s, 1 H, CH_2N of *E* isomer), 4.17 (s, 1 H, CH_2N of *Z* isomer), 4.8–4.7 (m, 4 H), 3.6–3.4 (m, 4 H).

1-(*tert*-Butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanone Oxime (Timolone Oxime, 5b). In a 250-mL round-bottomed flask fitted with a dropping funnel was placed **11b** (9.8 g, 33.5 mmol) in THF (147 mL). The solution was cooled in an ice bath, and a solution of *tert*-butylamine (12.2 g, 168 mmol) in THF (20 mL) was added through the dropping funnel during 10 min, with the reaction temperature kept at −5 to 0 °C (pH 6–7). The stirring was continued for 1 h at the same temperature. The solvent was evaporated under reduced pressure at 25 °C. To the residue was added diluted HCl (2.8 ml of concentrated HCl), and it was extracted with ethyl acetate. To the aqueous layer separated was added a dilute $NaHCO_3$ solution ($NaHCO_3$, 3.1 g) at −5 °C (pH ~6–7). It was extracted with ether to remove some impurities. Small amounts of $NaHCO_3$ (0.1–0.3 g) were added to the aqueous layer to make it slightly basic, and the mixture was extracted with ether. This procedure was repeated four times (pH was about 8) and another three times after the pH was raised to about 9 with dilute NaOH solution. The organic extracts were combined, washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo, yield 3.1 g (28%). The crude product was triturated with isopropyl ether to yield 2.8 g. This was recrystallized from isopropyl ether to yield 1.8 g (16%) of pure compound.

1-(*tert*-Butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanone Oxime Oxalate (Timolone Oxime Oxalate, 12b). In a 50-mL round-bottomed flask was placed a solution of oxalic acid (0.20 g, 2.22 mmol) in ether (10 mL). To this solution was added a solution of **5b** (0.43 g, 1.3 mmol) in ether. The mixture was stirred at room temperature for 1 h. The white nonhygroscopic crystals were filtered and dried in vacuo: yield 0.53 g (97%); mp 165–166 °C dec; NMR ($CDCl_3$) δ 5.3 (s, 2 H), 3.9–3.7 (m, 4 H), 3.6–3.4 (m, 6 H), 1.1 (s, 9 H). Anal. ($C_{18}H_{25}O_7N_5$) C, H, N.

5-(3-Chloro-2-hydroxypropoxy)-3,4-dihydrocarbostyril (8c). In a 250-mL round-bottomed flask fitted with a reflux condenser were placed 5-hydroxycarbostyril (15.0 g, 92 mmol), epichlorohydrin (34.2 g, 0.37 mol), morpholine (1.5 mL), and dioxane (90 mL). The mixture was refluxed for 16 h, and then it was concentrated in vacuo (20 mmHg) at 80–90 °C. To the residue was added 300 mL of 2 N HCl, the mixture was stirred for 15 min, then 0.8–1.0 L of ethyl acetate was added, and the mixture was stirred vigorously for 0.5 h. The organic layer was separated, washed well with water, then with dilute $NaHCO_3$ solution, and again with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo: yield 19.9 g (85%); NMR (DMSO-d_6) δ 10.3 (s, 1 H, NH), 7.25–6.50 (m, 3 H, Ph), 4.20–3.60 (m, 5 H, OCH_2CHCH_2Cl), 3.00–2.30 (m, 4 H, CH_2CH_2CO).

5-(3-Chloro-2-oxopropoxy)-3,4-dihydrocarbostyril (10c) was synthesized according to the method described for **10a**: NMR (DMSO-d_6) δ 10.10 (s, 1 H, NH), 7.20–7.00 (m, 1 H, Ph), 6.65–6.50 (m, 2 H, Ph), 4.95 (s, 2 H, OCH_2), 4.70 (s, 2 H, CH_2Cl), 3.0–2.8 (m, 2 H, CCH_2CO), 2.5–2.3 (m, 2 H, CH_2CCO).

5-[3-Chloro-2-(hydroxyimino)propoxy]-3,4-dihydrocarbostyril (11c) was synthesized similarly to the method give for **11b**: NMR (DMSO-d_6) δ 11.88 (s, 0.3 H, NOH of *E* isomer), 11.80 (s, 0.7 H, NOH of *Z* isomer), 10.08 (s, 1 H, NH), 7.30–6.50 (m, 3 H, Ph), 4.93 (s, 1.4 H, OCH_2 of *Z* isomer), 4.73 (s, 0.6 H, OCH_2 of *E* isomer), 4.38 (s, 2 H, CH_2Cl of *Z* and *E* isomers), 3.00–2.80 (m, 2 H, CCH_2CO), 2.65–2.40 (m, 2 H, CH_2CCO).

5-[3-(*tert*-Butylamino)-2-(hydroxyimino)propoxy]-3,4-dihydrocarbostyril (Carteolone Oxime, 5c). In a 100-mL round-bottomed flask fitted with a dropping funnel were placed **11c** (2.0 g, 7.45 mmol) and THF (70 mL). The solution was cooled to 0 °C, and a solution of *tert*-butylamine (0.82 g, 1.17 mL, 11.2 mmol) in THF was introduced through the dropping funnel. The mixture was stirred with cooling for 2 h. To the reaction mixture was added a solution of oxalic acid (1.48 g, 16.4 mmol) in THF. The precipitate was filtered, triturated with water (600–700 mL) by stirring well for 15 min, and filtered again. The filtrate was extracted with ethyl acetate several times. The aqueous layer was cooled to 0 °C, basified with a dilute $NaHCO_3$ solution ($NaHCO_3$, 0.81 g), and immediately extracted with ethyl acetate. The extract was evaporated in vacuo (20 mmHg) at 30 °C, yield 0.63 g (28%). Recrystallized from 2-propanol, the product was the *E* isomer: mp 177–180 °C dec; NMR (DMSO-d_6) δ 11 (s, 1 H), 10.1 (s, 1 H), 7.3–7.1 (m, 1 H), 6.7–6.5 (m, 2 H), 4.9 (s, 2 H), 3.3 (s, 3 H, contain NH), 3.0–2.8 (m, 2 H), 2.6–2.3 (m, 2 H), 1.05 (s, 9 H).

5-[3-(*tert*-Butylamino)-2-(hydroxyimino)propoxy]-3,4-dihydrocarbostyril Hydrochloride (Carteolone Oxime Hydrochloride, 12c). The free base **5c** was converted to the hydrochloride salt **12c** in ether with HCl gas, mp 167–169 °C dec. Anal. ($C_{16}H_{24}O_3N_3Cl$) C, H, N.

1-(Isopropylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanone Oxime (5d). In a 200-mL round-bottomed flask were placed 3-chloro-1-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanone oxime (**11b**) (3.53 g, 12.1 mmol), isopropylamine (3.56 g, 60.3 mmol), and THF (71 mL). The mixture was stirred at room temperature for 2.5 h. The reaction mixture was concentrated in vacuo at room temperature. The residue as triturated with isopropyl ether, and precipitated crystals were filtered with suction. The crystals were dissolved in dilute HCl solution. To the solution were added ether and $NaHCO_3$ in small portions under vigorous stirring conditions. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo: yield 0.42 g (11.6%) from isopropyl ether.

1-(Isopropylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanone Oxime Hydrochloride (12d). The oxime **11b** (0.25 g) was dissolved in ether, and ether saturated with HCl was introduced dropwise into the solution. The mixture was stirred for 10 min, filtered, and dried in vacuo overnight, yield 89%. Anal. ($C_{12}H_{22}N_5O_3SCl$) C, H, N.

Pharmacology. Effect on the Intraocular Pressure (IOP) of Rabbits. Adult male New Zealand albino rabbits weighing 2.5–3.5 kg were used. Animals were kept in individual cages with free access to food and water. Intraocular pressure was measured by using a Digilab Model 30 R pneumatonometer. The pneumatonometer readings were checked at least twice a day by using the Digilab calibration verifier. All measurements were obtained from unrestrained, unanesthetized rabbits. One drop of 0.5% propacaine (Ophthetic-Allergan Pharmaceuticals, Inc.) diluted 1:2 with saline was instilled in each eye immediately prior to IOP measurement. Drugs were administered as 1% or 2.5% solution in buffer pH 7.4 or in saline in both eyes of a group of at least four rabbits. Another group of at least three rabbits served as control and was administered the carrier only. IOP was recorded after 30 and 60 min and then after 2, 3, 4, 6, and 8 h following the drug or the carrier administration. All IOP measurements reported were carried out by the same operator using the same tonometer. Values are given as means ± standard error (SE) of the mean. Significance of the change was determined by using the Student's *t* test.

Effect on Resting Heart Rate and on Isoprenaline-Induced Tachycardia in Rats. Groups of seven male Sprague–Dawley rats weighing 150–250 g were used. Each animal was anesthetized with sodium pentobarbital (50 mg/kg), and the jugular vein was cannulated with PE50 tubing. This cannula was subcutaneously threaded around the neck and exteriorized dorsally. The cannula was filled with heparin solution (1000/μL) and sealed with a solid 22-gauge stylet. Animals were housed in individual stainless steel cages, and at least 24 h was allowed for recovery from the surgery. Food and water were provided ad libitum. On the day of the experiment, the heart rate of each rat was monitored with a plethsmograph (Buffington clinical devices) and the data recorded on a Physioscribe II recorder (Stoelting Co.). One hour was allowed as an equilibration period before any drugs were administered. Drugs were dissolved in normal saline as 0.3% solution and were administered intravenously at a dose of 6 mg/kg. The resting heart rate was then recorded after 1, 3, 5, 10, and 15 min following iv injection. Isoprenaline (isoproterenol bitartrate, Sigma Co.) was then administered subcutaneously at a dose of 50 μg/kg, and the heart rate was recorded for 3, 5, 10, 15, 20, 30, 45, and 60 min after administration. A control group of seven animals was intravenously administered saline solution and was treated exactly in the same manner as the drug-treated groups.

In another set of experiments, the effect of the oral administration of propranolol hydrochloride (**1a**) and propranolone oxime hydrochloride (**12a**) in doses of 25, 50, and 100 mg/kg on the resting heart rate and isoprenaline tachycardia was evaluated in rats. Drugs were administered to groups of five rats by using a stomach tube, and the heart rate was recorded for 1 h. Then isoprenaline (50 μg/kg, sc) was administered, and the heart rate was recorded after 3, 5, 10, 15, 20, 30, 45, and 60 min following administration. A control group of five rats was treated exactly in the same manner after the oral administration of the appropriate volume of saline solution.

Significance of the difference between the effect of saline solution and the drugs under investigation on the resting heart rate and on isoprenaline tachycardia was analyzed by using the Student's *t* test. Values are given as mean ± SE of the mean.

In Vivo Distribution: Metabolism Studies. A. In Ocular Tissues of Rabbits. Groups of at least four adult male New Zealand albino rabbits weighing 2.5–3.5 kg were used. Standard doses of 100 μL of a 1% solution of the drugs in saline solution were administered topically to both eyes of each rabbit. After appropriate time intervals (30, 60, and 120 min), animals were sacrificed. Aqueous humor was obtained by making a single puncture at the limbus by using a 25 gauge × $^5/_8$ in. needle attached to a 1-cm^3 syringe. Then the cornea and the iris–ciliary body were isolated. The tissues were pooled and homogenized by using a Tekmar SDT tissuemizer in ice cold perchloric acid (0.05 M) which contained 0.05% sodium metabisulfite as antioxidant. Samples were then rehomogenized in CH_3OH to prepare 10% homogenates, transferred to microfilters, and centrifuged for 20 min at 10000 rpm to precipitate proteins. Aqueous humor was analyzed as such without any further dilution. Aliquots of 5–20 μL of the 10% tissue homogenate samples were analyzed by HPLC. Quantitation was done by using a calibration curve obtained by the addition of known amounts of the compound to aqueous humor, iris–ciliary body, or cornea obtained from a control rabbit after topical administration of saline solution.

B. In Rat's Blood. A group of seven adult male Sprague–Dawley rats weighing 150–250 g was used. Animals were intrajugularly injected with propranolone oxime hydrochloride (**12a**) at a dose of 6 mg/kg. After 1, 3, 5, 20, 40, and 60 min, 1 mL of blood was withdrawn from the jugular vein and dropped immediately into a tared tube containing 1 mL of ice-cold acetonitrile. The tubes were vigorously shaken, centrifuged, decanted, and analyzed for propranolol (**1a**) and propranolone oxime (**5a**) by HPLC. Quantitation was done by using a calibration curve obtained by addition of known amounts of propranolone oxime hydrochloride (**12a**) to blood obtained from a control rat pretreated with saline solution.

Analytical Method. A high-pressure liquid chromatography (HPLC) method was developed for the assay of the β-blockers and their ketoxime analogues in biological fluids. The chromatographic analysis was performed on a system consisting of Beckman Model 112 solvent delivery system, Model 340 Injector, and Waters Model 481 variable wavelength LC spectrophotometer. An ASI reverse-phase Chrompack C_{18} column, operated at ambient temperature, was used for all separations. The mobile phase used for separation of propranolol (**1a**) and propranolone oxime (**5a**) consisted of water (90 mL), 1-heptanesulfonic acid (1 g), 0.1 M acetic acid (10 mL), 0.1 M triethanolamine (100 mL), and methanol (799 mL). With a flow rate of 1.5 mL/min, the two compounds showed retention times of 2.44 and 3.21 min for propranolone oxime and propranolol, respectively. The mobile phase used for separation of carteolol (**1c**), carteolone oxime (**5c**), timolol (**1b**), timolone oxime (**5b**), and the *N*-isopropyl analogue of timolone oxime (**5d**) consisted of water (430 ml), 1-heptanesulfonic acid (2 g), 0.1 M acetic acid (40 mL), tetrahydrofuran (30 mL), 0.1 M triethanolamine (100 mL), and methanol (398 mL). With a flow rate of 1.5 mL/min, the retention times for these compounds were 3.10, 3.54, 6.10, 7.22, and 9.15 min for carteolone oxime (**5c**), carteolol (**1c**), the *N*-isopropyl analogue of timolone oxime (**5d**), timolone oxime (**5b**), and timolol (**1b**), respectively.

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