Avoidance of "First-Pass" Elimination of Rectally Administered Propranolol in Relation to the Site of Absorption in Rats

Leo G. J. de Leede[1,3], Albertus G. de Boer[1], Jacques P. J. M. Havermans[1], and Douwe D. Breimer[2]

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Abstract: The extent of "first-pass" elimination of racemic propranolol and dextropropranolol in doses of 0.25 or 0.50 mg was investigated in relation to the site of drug administration in the rectum of rats. The compounds were given orally, i.v., and rectally at distances of 2 and 1 cm from and directly at the anus by low volume zero-order 30 min infusion. Unchanged propranolol was determined in blood, and propranolol and three metabolites were measured in urine. The systemic availability of propranolol after oral administration was approximately 6 %. Rectal administration at 2 cm, at 1 cm and directly at the anus (0.2 cm) gave two, three and six times higher values, respectively. The more distal application site produced urinary metabolite profiles that were comparable to those observed after oral administration, while application directly at the anus was similar to i.v. dosing. In all experiments log-linear elimination phases with comparable elimination half-lives (range 12–18 min) were found, except with the 0.50 mg dose after i.v. and rectal administration close to the anus which showed a non-linear profile. The mean systemic availability after rectal administration of 0.25 mg dextro-propranolol close to the anus was 50 and 64 % as compared to a 0.25 and 0.125 mg i.v. dose, respectively. The rectal route may be used for propranolol to partially prevent hepatic first-pass metabolism. However, avoidance of presystemic elimination is maximal only in the immediate vicinity of the anus as the venous blood supply of the upper part of the rectum of rats appears to be connected to the portal system and the lower part to the general circulation.

Propranolol exhibits significant hepatic "first-pass" elimination after oral administration (1, 2). Intravenous administration can be used to avoid this effect, but the rectal route may also be promising in this respect (3). In human subjects systemic availability of rectally administered lidocaine was twice as much when compared to oral administration (4). In rats rectal administration of both lidocaine and propranolol solutions resulted in a considerable increase in systemic availability compared to oral administration (5, 6). Recently we demonstrated for lidocaine that the site of drug administration in the rectum is a predominant factor that influences the extent of avoidance of "first-pass" elimination (7). When administered directly at the anus of the rat lidocaine availability was 72 % as compared to i.v. administration, whereas availability was reduced to 12 % at 4 cm from the anus. It is likely that the venous drainage of the rectum of the rat is comparable to that in man, where the middle and lower rectal veins pass directly into the vena cava, whereas the upper one is connected to the portal system.

[1] Department of Pharmaceutical Technology and Biopharmacy
[2] Department of Pharmacology University of Leiden, The Netherlands
[3] Correspondence Dr. L. G. J. de Leede
present adress Pharm. R & D Organon Int., P.O. Box 20, 5340 BH Oss, The Netherlands.

In the present study site-specific rectal propranolol administration was investigated in relation to its systemic availability in order to assess the degree of avoiding "first-pass" elimination relative to oral and i.v. administration. Propranolol in doses of 0.125 to 0.50 mg was given orally, i.v. and rectally at 2 and at 1 cm from the anus and close to the anus (approximately at 0.2 cm) of the rat by low-volume infusion during 30 min to avoid spreading. For the three different routes of drug administration experiments were performed with the racemic mixture and with dextro-propranolol to exclude a possible systemic pharmacodynamic effect. In addition to the assay of propranolol blood levels, the metabolite profile in urine was measured in order to determine the relationship between metabolism and route and site of drug administration.

Materials and Methods

Animals and Blood Sampling Technique

Male Wistar rats, weight 150 to 175 g (mean 167 ± 10 g), were used. Six rats were used for each type of experiment, and each rat participated once. The rats were fasted overnight in a metabolic cage with free access to water. Between 9.00 and 10.00 a.m. on the day of the experiment, the animals were cannulated in the right common carotid artery in caudal direction. Under light ether anesthesia the pvc cannula (length 50 cm, o.d. 1.0 mm, i.d. 0.5 mm, Talas, Ommen, The Netherlands) filled with heparinized saline (200 I.U./ml) was inserted and, to avoid destruction by the rat, was pulled s.c. emerging on the nape of the neck. The animals were allowed to move freely; urine and blood samples were collected without touching the animals. Blood samples of 0.100 ml were taken at 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 270, and 300 min. After each sample the cannula was filled with heparinized saline to prevent clotting. The samples were mixed immediately with 1.0 ml water (0°C) and kept at −20°C until assayed. Urine was collected for 24 h after drug administration in a solution of ascorbic acid (50 mg/ml), diluted with water to 100.0 ml and kept at −20°C.

Drug Solution

The drug solution contained 0.125, 0.25 or 0.50 mg of (racemic) propranolol HCl or dextro-propranolol HCl, calculated as the base, in 50 µl of distilled water. Propranolol and dextro-propranolol were generously supplied by I.C.I. Ltd. (Macclesfield, Cheshire, U.K.). For the i.v. and rectal experiments the 50 µl solution was used, but for the oral experiments the solution was diluted to 500 µl with distilled water.

Drug Administration

Approximately 90 min after cannulation and insertion of the rectal infusion system, the drug administration was started. For oral administration the rats were given 0.50 mg propranolol via a stomach tube. For i.v. administration a cannula was introduced into the right external jugular vein after cannulation of the carotid artery for blood sampling. Doses of 0.25 and 0.50 mg propranolol and 0.125 and 0.25 mg dextropropranolol were given i.v. by zero-order rate infusion over 30 and occasionally 60 min. For rectal administration an infusion system was installed into the animals. The system consisted of a cannula with one end pulled s.c. and emerging on the nape of the neck and connected to the infusion system. The other end was introduced into the rectum and was surrounded in the anus by a septum (the tip of a 1 ml disposable syringe), which was ligated with a thread introduced s.c. around the anus to prevent expulsion. The distance from the midpoint of the septum to the end of the cannula in the rectum was 2 or 1 cm. A cannula with a septum-shaped piece of teflon at the anal end was used for propranolol administration as closely as possible to the anus of the rat. At 2 and 1 cm from the anus 0.50 mg propranolol was given, and directly at the anus (at approximately 0.2 cm) 0.50 and 0.25 mg propranolol and 0.25 mg dextro-propranolol. Rectal administration took place by zero-order rate infusion for 30 min.

Care was taken that propranolol was not absorbed to the cannulas and tubes nor to the septum in the anus, by using inert materials and an *in vitro* check procedure.

Assay of Propranolol and Metabolites in Blood and Urine

To the mixture of 100 µl blood and 1.0 ml water, or an aliquot of urine, the internal standard, 4 methyl-propranolol, and 50 µl (50 mg/ml) ascorbic acid as antioxidant were added. This was mixed with 1.0 ml buffer pH 9.5 (1M sodium carbonate and sodium bicarbonate) and extracted twice with 3.0 ml ethyl acetate during 15 sec on a whirlmixer. After centrifugation for 5 min at 2500 ×g, the upper organic layers were transferred to a conical evaporation tube containing 200 µl sulfuric acid (0.25 N) and ascorbic acid (25 mg/ml). The mixture was extracted for 15 sec on a whirlmixer and centrifuged for 5 min at 2500 ×g. The upper organic layer was discarded and the aqueous layer was completely cleared from the ethyl acetate by evaporation at 50°C under reduced pressure in a Buchler Vortex Evaporator. An aliquot of the acid water layer was injected directly into the HPLC. The HPLC consisted of a Waters M-45 solvent delivery system, an automatic injection system (WISP model 710 B) and a Perkin Elmer 204 Fluorescence detector. A µ-Bondapack C18 (Waters) column was used, and the eluent consisted of phosphoric acid 0.06 % : acetonitrile = 70 : 30 (V/V). The excitation wavelength was 303 nm and the emission wavelength 335 and 405 nm for propranolol and 4 OH-propranolol (4 OH-P), respectively. Linear calibration curves were obtained for propranolol between 1 and 1000 ng/ml and for 4 OH-P between 5 and 1000 ng/ml blood or urine. For the determination of conjugated metabolites, propranolol glucuronide (P-G) and 4 OH-propranolol glucuronide (4 OH-P-G) were enzymatically hydrolyzed and analyzed as propranolol and 4 OH-P. Limpet Acetone powder (Sigma, St. Louis, USA) containing β-glucuronidase, was used for 90 min at pH 5.5 and at 37°C to achieve complete deconjugation.

Calculations and Pharmacokinetic Analysis

The elimination half-lives of propranolol were determined by least-square regression analysis from the log-linear terminal parts of the blood concentration time curves. The areas under the curves (AUC) were calculated according to a method described by Chiou (8). The AUC from t = 0 to the maximum concentration was calculated with the linear, and after the maximal concentration with the logarithmic trapezoidal rule to infinity.

The mean absolute systemic availability (F) of propranolol was calculated by:

$$F = \frac{AUC}{AUC_{i.v.}} \times z\frac{D_{i.v.}}{D} \times 100\,\% \qquad (1)$$

in which $AUC_{i.v.}$ and AUC are the area under the curve of the i.v. and oral or rectal experiments, respectively, and D is dose.

For statistical evaluation of the results a one-way ANOVA (analysis of variance) was used. The S-method of Scheffé (9) with $\alpha = 0.05$ was used in multiple comparison analysis. When highly significant differences in standard deviations made the use of ANOVA inappropriate, the non-parametric test of Wilcoxon (10) was used in comparing two different groups of data. Differences were assumed to be significant when $p < 0.05$.

Results

Fig. 1 shows the mean propranolol blood concentration-time curves during and after i.v. and rectal (0.2 cm from the anus) administration of 0.50 mg propranolol by 30 min infusion. After the infusion first a distribution phase can be recognized, in both cases followed by a non-linear elimination profile. When the dose was decreased to 0.25 mg, this non-linearity in the elimination phase disappeared as is shown in Fig. 2. Since

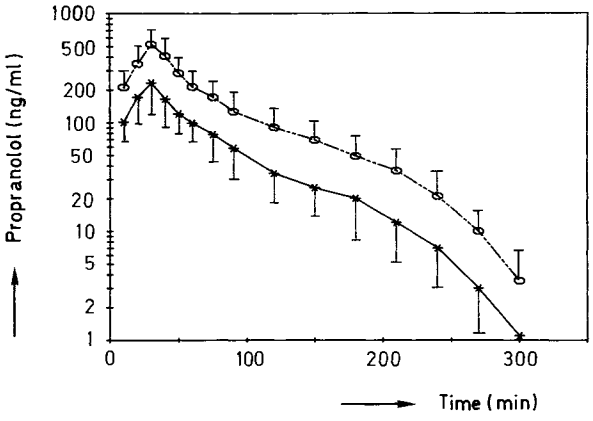

Fig. 1 Mean blood concentration versus time curves of propranolol after i.v. (o) and rectal administration close to the anus (✳) of 0.50 mg propranolol (n = 6 in both experiments; bars represent S.D.).

distribution and elimination both occur relatively rapidly, the separate phases cannot be distinguished in the concentration-time curves of Fig. 2.

The mean curves of the rectal propranolol experiments at 2 and at 1 cm from the anus (dose 0.50 mg) and directly at the anus (dose 0.25 mg) are shown in Fig. 3 together with the mean oral curve (dose 0.50 mg). A non-linear elimination phase was not observed in any of these experiments. Compared to oral administration higher blood concentrations are found when propranolol is administered rectally. In the sequence of rectal administration at 2 cm, at 1 cm, and at 0.2 cm from the anus,

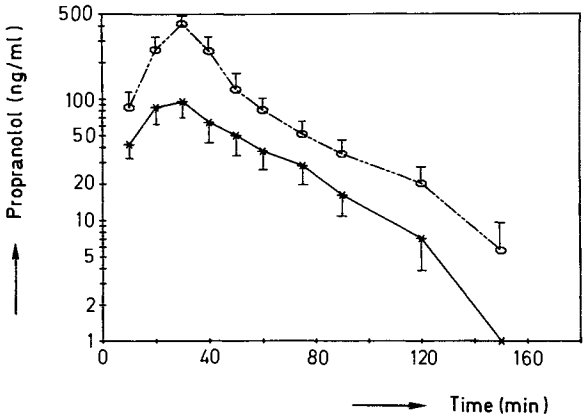

Fig. 2 Mean blood concentration versus time curves of propranolol after i.v. (o) and rectal administration close to the anus (✳) of 0.25 mg propranolol (n = 6 in both experiments; bars represent S.D.).

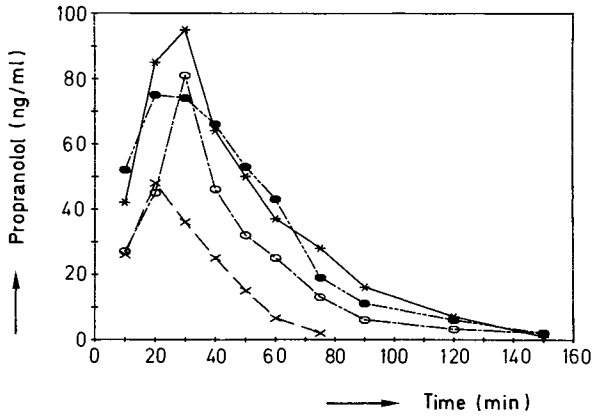

Fig. 3 Mean blood concentration versus time curves of propranolol after oral (×) and rectal administration at 2 cm (o) and 1 cm (•) from the anus of 0.50 mg propranolol and rectal administration at 0.2 cm (✳) from the anus of 0.25 mg propranolol (n = 6 in all experiments).

the propranolol concentrations increased considerably, particularly if the lower dose at 0.2 cm from the anus is taken into account. Table I gives the elimination half-lives and AUC of the oral, rectal and i.v. experiments. The mean elimination half-lives ranged from 12–18 min and were not significantly different. For the i.v. and rectal experiments at 0.2 cm from the anus with 0.50 mg propranolol exact half-lives could not be determined because of non-linearity in the elimination phase (Fig. 1). Total AUC increased when the drug was delivered more closely to the anus. The rectal AUC values all differed significantly from each other. The i.v. values were always higher, whereas oral administration consistently resulted in lower values. The systemic availability, as calculated by equation 1, of the propranolol experiments with the linear elimination profile relative to the 0.25 mg i.v. value is also given in Table I. At 2 cm from the anus the systemic availability is twice as much as the oral availability. At 1 cm from the anus a threefold increase, and directly at the anus a sixfold increase in availability relative to oral administration was found.

In Table II the results of drug and metabolite analysis in urine are shown for the propranolol experiments with linear elimination profile. Mean total recovery of propranolol plus the measured metabolites varied between 18 and 34 %, calcu-

Table I. Elimination half-lives, AUC and systemic availability (F) after oral, rectal and i.v. administration of propranolol to rats (mean ± S.D.; n=6 in all experiments).

	Dose (mg)	Elimination Half-live (min)	AUC (μg.min.ml^{-1})	F (%)
Propranolol				
i.v.	0.50	✳	29.0 ± 6.9	–
rectal at 0.2 cm	0.50	✳	15.6 ± 3.8	–
oral	0.50	12 ± 3	1.6 ± 0.5	6
rectal at 2 cm	0.50	14 ± 7	3.0 ± 1.0	12
rectal at 1 cm	0.50	17 ± 5	4.4 ± 0.9	17
rectal at 0.2 cm	0.25	18 ± 1	4.7 ± 1.4	37
i.v.	0.25	17 ± 2	12.6 ± 1.6	100
Dextro-propranolol				
rectal at 0.2 cm	0.25	17 ± 3	4.9 ± 1.9	50 64
i.v.	0.25	16 ± 2	9.7 ± 0.7	100
i.v.	0.125	16 ± 2	3.8 ± 0.1	100

* could not be determined adequately due to non-linearity in the elimination phase (Fig. 1)

lated as percentage of the dose. The lower value corresponds to i.v. administration and the higher to oral administration, while the recovery following rectal administration was in between. Comparing the metabolite concentrations, all rectal values fall in between the corresponding oral and i.v. data. The values after i.v. administration agree best with those obtained after rectal administration close to the anus.

Intravenous and rectal experiments close to the anus were performed with the β-receptor blockade inactive dextro-isomer, to exclude the influence of possible systemic dynamic effects on the AUC and hence on the evaluation of the degree of avoidance of presystemic elimination. In Fig. 4 the mean curves after rectal administration of dextro-propranolol 0.2 cm from the anus (dose 0.25 mg), i.v. 0.25 mg infused over 30 min and 0.125 mg infused over 60 min are given. Intravenous infusion of the low dose over 60 min resulted in propranolol concentrations comparable to those following rectal administration. The elimination half-lives and AUC are given in Table I. Elimination half-lives were the same after dextro-propranolol compared to the experiments performed with racemic

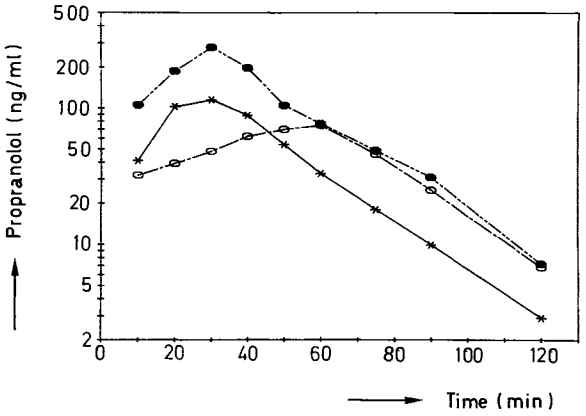

Fig. 4 Mean blood concentration versus time curves of propranolol after i.v. (•) and rectal administration at 0.2 cm from the anus (✳) of 0.25 mg dextro-propranolol over 30 min and i.v. (o) of 0.125 mg over 60 min (n = 6 in all experiments).

Table II. Urinary recovery of propranolol and its metabolites after oral, rectal and i.v. administration of propranolol to rats (mean ± S. D.; n = 6 in all experiments).

	Dose (mg)	Amount in urine calculated as % of the dose				
		Propranolol	Propranolol glucuronide	4-hydroxy-propranolol	4-hydroxy-propranolol glucuronide	Total
Propranolol						
oral	0.50	0.3 ± 0.1	0.5 ± 0.4	3.3 ± 1.1	30 ± 12	34 ± 11
rectal at 2 cm	0.50	0.6 ± 0.3	0.9 ± 0.7	0.5 ± 0.2	25 ± 5	27 ± 6
rectal at 1 cm	0.50	0.6 ± 0.2	1.2 ± 1.2	0.3 ± 0.4	23 ± 15	25 ± 16
rectal at 0.2 cm	0.25	1.8 ± 0.2	4.5 ± 1.1	n.d.	19 ± 2	25 ± 2
i.v.	0.25	2.5 ± 0.6	4.0 ± 0.8	n.d.	12 ± 4	18 ± 4
Dextro-propranolol						
rectal at 0.2 cm	0.25	0.5 ± 0.4	2.0 ± 0.9	n.d.	18 ± 4	21 ± 4
i.v.	0.25	0.5 ± 0.3	1.5 ± 1.0	n.d.	9 ± 4	11 ± 4
i.v.	0.125	0.5 ± 0.1	3.2 ± 0.2	n.d.	15 ± 1	18 ± 1

n.d.: not detectable

propranolol. The same is true for the AUC obtained after rectal administration, but the AUC of the i.v. experiment is significantly smaller compared to the corresponding racemic experiment. The systemic availability of rectally administered dextro-propranolol near the anus is 50 and 64 % relative to the 0.25 and 0.125 mg i.v. dose, respectively. The urinary excretion data of propranolol and metabolites after administration of dextro-propranolol are given in Table II. Significantly less parent drug and its glucuronide were found with the dextro-isomer. The relative amount of 4-hydroxypropranolol glucuronide formed after i.v. administration of dextro-propranolol was significantly increased when the dose was decreased from 0.25 to 0.125 mg and the infusion time was twice as long. The urinary excretion data after rectal administration of dextro-propranolol at 0.2 cm from the anus agree best with those obtained with the i.v. 0.125 mg dose.

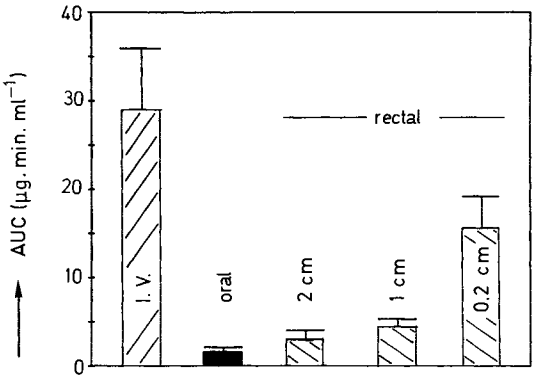

Fig. 5 The relationship between AUC and the site of administration in the rectum compared to oral and i.v. administration of 0.50 mg propranolol (n = 6 in all experiments; bars represent S.D.).

Discussion

In the present study the relationship between systemic availability and the site of rectal propranolol administration in rats was investigated. The results confirm those obtained in a previous study with lidocaine, namely, that the degree of avoidance of "first-pass" elimination is indeed strongly dependent on the site of rectal drug administration (7, 11).

In Fig. 5 the AUC's for the oral, i.v. and three rectal experiments are given after administration of 0.50 mg propranolol to visualize the rank order in increasing systemic availability.

Administration of propranolol directly at the anus, by low volume zero-order rate infusion to avoid spreading, led to the highest avoidance of presystemic elimination. More distally to the anus, the drug's availability decreased towards the value observed after oral dosing. This conclusion is not only based on comparative AUC values for the different sites of propranolol administration, but it is also supported by comparing the urinary excretion profiles (Table II). The amounts of free and conjugated propranolol are low after oral and much higher after i.v. administration, whereas the reverse is true for 4-hydroxypropranolol glucuronide (4 OH-P-G). The amounts of free and conjugated propranolol and 4 OH-P-G after rectal administration were in between the corresponding oral and i.v. values. While the systemic availability after oral administration of propranolol was only 6 %, rectal administration at 2 cm, at 1 cm, and directly at the anus gave 12, 17 and 37 %, respectively. Absorption of propranolol after rectal administration was probably complete because propranolol concentrations in blood started to decline immediately after drug administration was stopped, and furthermore, because the total recovery of metabolites excreted in urine after rectal administration was in between the values obtained in the oral and i.v. experiments. Published data on urinary recovery after propranolol administration to rats are scarce; free and conjugated 4-hydroxypropranolol was reported to account for 25 % of the dose after i.p. administration, which is in between the oral and i.v. data of the present investigation (12).

For a quantitative evaluation of the extent of liver bypass following rectal propranolol administration non-linear kinetics should be avoided. In Fig. 1 it was clearly shown that i.v. and rectal propranolol administration of 0.50 mg near the anus resulted in non-linear elimination kinetics, which was not observed after oral and rectal administration at 2 cm and at 1 cm from the anus. To avoid non-linear kinetic phenomena,

the propranolol concentration should not exceed approximately 150 ng/ml (13, 14, 15). Since relatively high propranolol concentrations could have an effect on liver bloodflow because of its β adrenergic receptor blocking properties, which might complicate a quantitative interpretation of the results, experiments were also performed with the pharmacologically inactive dextro-isomer. The mean systemic availability after administration of dextro-propranolol at 0.2 cm from the anus was 50 and 64 % relative to a 0.25 or 0.125 mg i.v. dose, respectively. Since the maximal blood concentration (Fig. 4) and the urinary excretion pattern (Table II) of the rectal administration are similar to the values obtained with i.v. administration of 0.125 mg, this latter experiment has been taken as the reference. The results with the dextro-isomer do not indicate that with the racemate the results are considerably affected by systemic effects of the active isomer.

The rectal route may be used for the high-clearance drug propranolol as a partially non-hepatic route, but avoidance of presystemic elimination is maximal close to the anus only. Previous studies on the rectal absorption of nitroglycerine (16) and lidocaine (7, 11) and the results of this study further support the hypothesis that the venous blood supply of the upper part of the rectum of rats is connected to the portal system and that the far lower part passes directly into the general circulation. This situation is quite comparable to that found in man.

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Improved Delivery Through Biological Membranes. XVII[3]. A Site-Specific Chemical Delivery System as a Short-Acting Mydriatic Agent

Nicholas Bodor[1,2] and Gary Visor[1]

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Abstract: O,O-Di(ethylsuccinyl) adrenalone was synthesized and studied as a potential short-acting mydriatic agent. This unsymmetrical tetraester has a very short hydrolytic half-life in biological fluids (approximately 1 minute). The hydrolysis produces the inactive adrenalone. On the other hand, a reduction-hydrolytic sequence resulting in adrenaline was established as the mechanism of action of these types of compounds. The facile activation to epinephrine and fast deactivation to adrenalone of the unreduced chemical delivery system results in a short-acting mydriatic agent, a potentially important diagnostic or surgical agent.

The unexpected and high ocular sympathomimetic activity of a series of diester derivatives of adrenalone (**1**) was recently described (2). While adrenalone is the synthetic precursor of epinephrine, it has very little intrinsic activity of its own (3). The simple diester derivatives of adrenalone, however, were found to produce dramatic mydriasis and decrease in the intraocular pressure following topical administration. The effect was established even with solution concentrations equi-

[1] Department of Medicinal Chemistry, Box J-4, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610
[2] To whom correspondence should be addressed.
[3] Part XVI of this series see reference (1).