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Prodrug Approaches to Enhancement of Physicochemical Properties of Drugs IV: Novel Epinephrine Prodrug

ANWAR HUSSAIN [x] and J. E. TRUELOVE

Abstract ☐ The synthesis and characterization of a prodrug that appears to overcome the problem of inefficient absorption of epinephrine through the lipoidal membranes of the eye are described. The enzymatic rate of regeneration of epinephrine from the prodrug was determined using a rabbit eye homogenate, rabbit plasma, and human plasma. The prodrug had no activity of its own when tested against a guinea pig smooth muscle preparation. Upon enzymatic regeneration of epinephrine from the prodrug, however, the reaction mixture exhibited α-adrenergic activity equivalent to that of epinephrine when tested in the same preparation.

Keyphrases ☐ Prodrugs—epinephrine prodrug synthesized, screened for adrenergic activity, rabbit eye homogenate and plasma, human plasma ☐ Epinephrine prodrug—synthesized, screened for adrenergic activity, rabbit eye homogenate and plasma, human plasma ☐ Adrenergic activity—epinephrine prodrug, screened in rabbit eye homogenate and plasma, human plasma

Previous reports in this series (1–3) presented examples of prodrug approaches that may be used to modify the physicochemical properties of drug molecules to reduce the gastric irritation of aspirin or to improve the dissolution characteristics of highly water-insoluble compounds such as allopurinol and phenytoin. This report presents an example demonstrating the utility of the prodrug approach in improving the efficiency of absorption of a highly polar molecule through lipoidal membranes.

BACKGROUND

Although epinephrine has been used for many years in eye drops for the management and treatment of glaucoma and in inhalant preparations for the treatment of bronchial asthma, problems arise with its use. One major problem is the occurrence of undesirable side effects, both ocular and systemic. McClure (4) recently listed some side effects resulting from topical applications of epinephrine.

In the treatment of glaucoma, relatively concentrated epinephrine solutions are instilled directly into the eye. However, because it is highly polar, little drug is absorbed. The remainder of the solution reaches the general circulation through the tear ducts, exerting its undesirable systemic side effects (5). Since many glaucomatous patients are over 40 years of age, some may have cardiac or circulatory disorders which could be aggravated by systemically absorbed epinephrine. Therefore, an improved form of epinephrine that would be effective at low concentrations seemed desirable.

The fundamental problem with epinephrine is its inefficient transport across lipoidal barriers due to its high polarity and low lipid solubility. It was felt that the transient blocking of the phenolic hydroxy groups would enhance the lipophilicity of epinephrine and significantly facilitate its absorption through the lipoidal membranes of the eye.

The synthesis of a novel prodrug of epinephrine (6), which has been shown to be approximately 100 times more effective clinically than epinephrine itself in the management of glaucoma (4), is reported here. Furthermore, the prodrug has been shown to be about 100–400 times weaker than epinephrine in affecting the cardiovascular systems of dogs and cats (4).

A successful epinephrine prodrug should be more lipophilic than epinephrine, possess adequate water solubility, regenerate epinephrine at a reasonable rate, and be stable enough to be formulated into conventional dosage forms. Furthermore, the blocking groups, upon cleavage, should have no toxicity of their own.

The prodrug, 3,4-dipivaloyloxy-α-(methylaminomethyl)benzyl alcohol perchlorate salt (I) (Scheme I) was a suitable candidate. Since the general pharmacology, toxicology, and clinical evaluation of the prodrug have already been reported (4), this article is concerned with the synthesis and *in vitro* enzymatic hydrolysis of the drug in a rabbit eye homogenate, rabbit plasma, and human plasma.

EXPERIMENTAL

Synthesis[1] of I—Fifty grams (0.27 mole) of α-chloro-3′,4′-dihydroxyacetophenone[2] (II) was dissolved in 200 ml of methanol. (Slight warming may be necessary to complete the solution.) Then 100 ml of

[1] The general synthetic procedure was presented in U.S. pat. 3,809,714.
[2] Obtained from a commercial source or synthesized by the reaction of pyrocatechol and chloroacetyl chloride in refluxing benzene.

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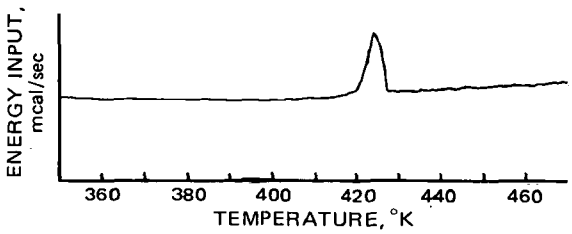


Figure 1—*Differential scanning calorimeter thermogram on heating of a sample of I.*

40% aqueous methylamine was slowly added, and the mixture was stirred at 50° for 2 hr (Scheme I). The solution was then stirred an additional 24 hr at ambient temperatures.

The crude α-methylamino-3′,4′-dihydroxyacetophenone precipitated from the reaction mixture and was recovered by filtration. After thorough washing with ether, the material was dissolved in 100 ml of 4 *N* HCl and 125 ml of methanol. After filtration through decolorizing charcoal, the product was precipitated as the hydrochloride salt (III) by the addition of seven parts of acetone. The salt, a white crystalline material, mp 242°, was used without further purification.

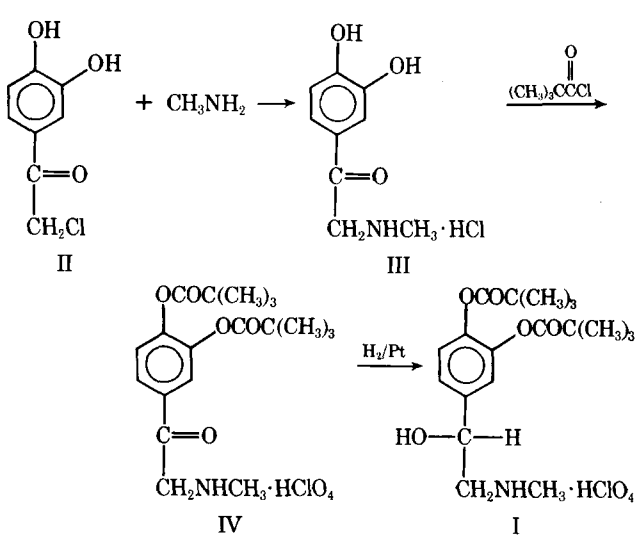
Compound III (25.3 g, 0.125 mole) was dissolved in 250 ml of ethyl acetate, and 0.125 mole of perchloric acid as the 70% aqueous solution was added with continuous stirring. Then 280 ml of pivaloyl chloride was added, and the mixture was slowly warmed to reflux. After 5 hr at reflux, the mixture was allowed to cool to room temperature with continuous stirring. The product, α-methylamino-3′,4′-(dipivaloyloxy)acetophenone (IV), was precipitated as the perchlorate salt by the addition of 500 ml of ether. The crude material was purified by dissolving in 75 ml of acetone and precipitating with water (150–200 ml).

Twenty grams (0.044 mole) of IV was dissolved in 300 ml of 95% ethanol in a 500-ml Parr reaction bottle. Then 1.5 g of platinic oxide was added, and the mixture was shaken under hydrogen at 50 psi for 1 hr. After filtration to remove the platinum, the solvent was removed on a rotary evaporator. The resulting oil was mixed with 200 ml of ether. The product (I) separated as a white crystalline material (mp 147–149°) when the ether solution was slowly added to an additional 1200 ml of vigorously stirred ether.

Anal.—Calc. for $C_{19}H_{30}ClNO_9$: C, 50.50; H, 6.69; Cl, 7.85; N, 3.10; O, 31.86. Found: C, 50.51; H, 6.40; Cl, 7.66; N, 3.11; O, 31.88.

The NMR, UV, TLC, and differential scanning calorimetry data were consistent with the proposed structure (I); NMR ($CDCl_3$): δ 6.6–7.4 (m, 5, aromatic and amino), 4.8–5.2 (m, 1, C*H*OH), 4.1–4.3 (d, 1, CHO*H*), 2.9–3.6 (m, 2, CHOHCH_2), 2.5–2.9 (s, 3, NCH_3), and 1.1–1.5 (s, 18, pivalyl); UV: 263 nm (ε 476); TLC [silica gel with chloroform–methanol–formic acid (30:10:1) and ninhydrin visualizer]: R_f 0.10 (epinephrine), 0.65 (I), and 0.80 (IV).

With differential scanning calorimetry, a single endotherm was observed at the melting point (421° K) of the compound, indicating



Scheme I

Table I—*In Vitro* Enzymatic Hydrolysis of the Prodrug

Minutes	Percent Hydrolysis in: Rabbit Plasma	Human Plasma	Rabbit Eye Homogenate
0	0	0	0
3	12.3	—	—
8	47.7	—	—
10	—	8.0	—
13	58.0	—	—
18	—	24.1	—
20	—	—	2.3
22	96.0	—	—
28	—	47.5	—
32	100	—	—
40	—	—	5.3
45	—	85.8	—
60	—	95.1	18.2
90	—	100	34.0
105	—	—	42.5
150	—	—	68.9
190	—	—	81.8
285	—	—	100

that the material was unitary. The endotherm remained unchanged upon cooling and reheating of the sample (Fig. 1).

Compound I is a racemic mixture and was resolved employing fractional crystallization of the dibenzoyl *d*-bitartrate salt. Sodium dibenzoyl *d*-bitartrate (8.4 g) was dissolved in 200 ml of methanol. Then 20 g of I was dissolved in 200 ml of methanol, and the solution was filtered and diluted with 400 ml of water. The sodium dibenzoyl *d*-bitartrate solution was added to the solution of I, and the total volume was adjusted to 840 ml with 50% aqueous methanol. The solution was cooled slowly (about 0.8°/hr) until the final temperature was 2.6°. The product was recovered by filtration and recrystallized from 50% aqueous methanol. Crystallizations were repeated until the product had a constant specific rotation of −26° and a melting point of 142–143°.

Analytical Procedure—A high-pressure liquid chromatograph[3] with a UV monitor (254 nm) was used. A 1-m × 2.1-mm i.d. stainless steel column, packed with a strong anion-exchange resin[4], was operated at 1200 psi and ambient temperatures, resulting in a flow rate of 1 ml/min. The mobile phase employed was a 0.01 *M* phosphate buffer at pH 7.4. Ten microliters of the appropriate solution (0.4 mg of prodrug/ml of plasma or eye homogenate) was injected and analyzed for epinephrine concentration by comparing the peak area to that of a standard epinephrine solution.

***In Vitro* Enzymatic Rate of Hydrolysis in Rabbit Eye Homogenate**—Upon sacrifice, the eyes of a New Zealand white rabbit were removed and washed thoroughly with cold isotonic saline solution to eliminate any traces of blood. The eyes were then homogenized in a ground-glass homogenizer. The homogenate was subjected to centrifugation for 10 min at 5000 rpm, yielding about 3 ml of a clear supernate. The supernate was transferred to a stoppered test tube and equilibrated to 37°.

After equilibration, a 2-ml sample of the preparation was placed in another tube, mixed with 0.2 ml of a 4-mg/ml aqueous solution of the prodrug, flushed with nitrogen, and maintained at 37°. Ten-microliter aliquots were periodically injected directly onto the liquid chromatographic column, and the liberation of epinephrine was followed as a function of time.

***In Vitro* Enzymatic Rate of Hydrolysis of Prodrug in Human or Rabbit Plasma**—Blood samples (5 ml) were withdrawn and centrifuged immediately. Two milliliters of the plasma was transferred to a stoppered test tube and equilibrated to 37°. After equilibration, 0.2 ml of the 4-mg/ml solution of prodrug was added and the mixture was maintained at 37°. Liberation of epinephrine was followed as already described.

Hydrolysis of Prodrug in Aqueous Humor of Rabbit Eyes—The aqueous humor was obtained from the rabbit eye by puncturing the anterior chamber with a needle and syringe. Fifty microliters of a 4-mg/ml aqueous solution of the prodrug was added to 0.5 ml of the

[3] Du Pont 820.
[4] Zipax SAX, du Pont.

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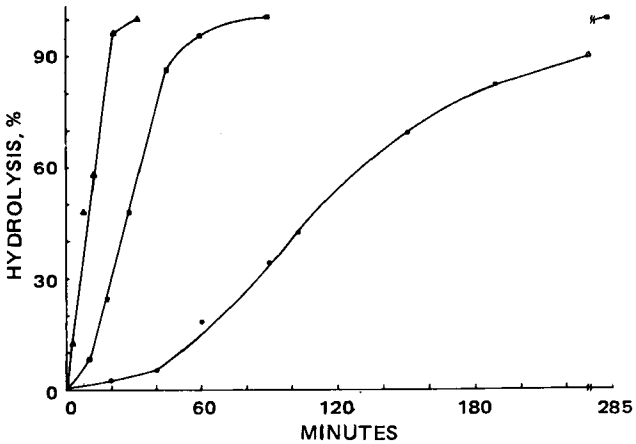

Figure 2—In vitro *enzymatic rate of hydrolysis of the prodrug at 37°. Key: ▲, rabbit plasma; ■, human plasma; and ●, rabbit eye homogenate.*

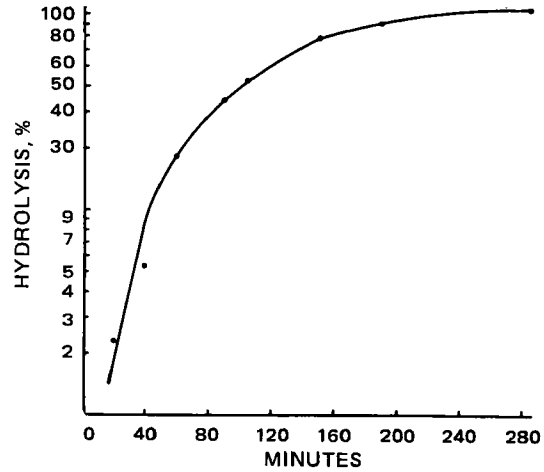

Figure 3—*Semilog plot of the rate of hydrolysis of the prodrug in the rabbit eye homogenate.*

aqueous humor, and the mixture was maintained at 37°. After 3 hr, the mixture was subjected to high-pressure liquid chromatographic analysis.

Effect of Prodrug on Guinea Pig Vas Deferens Preparation—To determine if the dipivaloyl derivative possesses pharmacological activity, its effect on the guinea pig smooth muscle was studied (7). The contraction of the guinea pig vas deferens was determined in the presence of epinephrine, the prodrug, and the product of complete hydrolysis of the prodrug in human plasma.

RESULTS AND DISCUSSION

The described synthetic procedure for the preparation of I appears satisfactory for the production of large batches of the prodrug. The same procedure was successfully applied to the preparation of the corresponding esters of isoproterenol (8) and phenylephrine (9).

Based on the reported clinical superiority of I in the management of glaucoma (4), this approach appears to be significant in improving the pharmacological activity of catecholamines. The fundamental problems with epinephrine, its high polarity and poor lipid solubility, appear to be overcome by the synthesis of the prodrug. In fact, the prodrug approach apparently is the only one capable of enhancing the pharmacological activity of epinephrine while reducing the incidence of its side effects. McClure (4) reported that the prodrug is about 100 times more effective than epinephrine in the management of glaucoma and about 100–400 times weaker than epinephrine in affecting the cardiovascular system of dogs and cats.

The lack of α-adrenergic activity of the prodrug was clearly demonstrated by its lack of activity in the guinea pig vas deferens preparation. However, the activity of epinephrine remained intact after hydrolysis of the pivaloyl groups in human plasma.

The prodrug is relatively stable toward hydrolysis in the absence of enzymes. However, in the presence of enzymes, the prodrug generates epinephrine at an acceptable rate. For example, the prodrug resists hydrolysis in the presence of aqueous humor but regenerates epinephrine in the total eye homogenate. This result may be due to the fact that enzyme levels are as much as 29 times higher in the lens tissue than in the aqueous humor (10).

The results of the hydrolytic study with the rabbit eye homogenate, rabbit plasma, and human plasma are shown in Table I and Fig. 2. These data show that the prodrug cleaves to epinephrine at a reasonable rate.

The *in vivo* cleavage rate may, in fact, be many times faster. A semilog plot of percent hydrolysis in rabbit eye homogenate *versus* time is shown in Fig. 3. It is apparent from these data that the rate of hydrolysis of the prodrug cannot be described by simple first-order kinetics.

Although other esters of epinephrine were made, the data indicate that the pivaloyl prodrug exhibits superior stability when compared to the corresponding acetate derivative (6). The pivalic acid generated upon hydrolysis of the prodrug exhibits a wide margin of safety, even when given orally in large doses (11).

Subsequent reports will investigate the effects of the pivaloyl blocking group on the activity of other catecholamines.

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