Relationship of *In Vitro* Hydrolysis of 17-Chloroacetylajmaline and 17-Acetylajmaline in Different Animal Species

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Abstract □ 17-Chloroacetylajmaline and 17-acetylajmaline are reported to have *in vivo* antiarrhythmic activity and are metabolized by hydrolysis. Since the hydrolysis product, ajmaline, may be the actual antiarrhythmic agent, the hydrolysis of these derivatives by various tissues of the guinea pig, rat, and mouse was determined *in vitro* by a titrimetric method and compared to hydrolysis by α-naphthylacetate. The heart is the most active tissue in the guinea pig for hydrolyzing 17-chloroacetylajmaline. The hydrolyzing activity is greater in the guinea pig than in rat or mouse heart, corresponding with the more significant pharmacological activity in the guinea pig. 17-Chloroacetylajmaline has a significantly lower K_m value than 17-acetylajmaline, which is in agreement with the *in vivo* activity.

Keyphrases □ 17-Chloroacetylajmaline—*in vitro* hydrolysis, relationship to hydrolysis of 17-acetylajmaline, different animal species □ 17-Acetylajmaline—*in vitro* hydrolysis, relationship to hydrolysis of 17-chloroacetylajmaline, different animal species □ Antiarrhythmic activity—*in vitro* hydrolysis of 17-chloroacetylajmaline and 17-acetylajmaline, different animal species

The presence of several esterases in the blood and body tissues may be important in the distribution and metabolism of drugs possessing an ester bond (1). The pharmacological effects also may be influenced, depending upon whether an active or an inactive moiety of the particular drug is released with this esterase activity.

This problem should be considered not only for the blood but also for the tissues, with particular reference to the target organ of drug action. Recently, a new ester of ajmaline (2–4), 17-chloroacetylajmaline, was proposed as a therapeutic agent for the treatment of arrhythmias[1]. Preliminary data showed that this new compound is more effective in guinea pigs than in other animal species such as rats and mice. Therefore, it was of interest to investigate comparatively the capacity of blood and tissues, with particular reference to the heart, of these animal species to hydrolyze 17-chloroacetylajmaline.

This study may show whether 17-chloroacetylajmaline is active *per se* or through formation of ajmaline. It may also give an explanation of the outstanding action seen in guinea pigs.

EXPERIMENTAL

Animals—Male Sprague–Dawley rats (200–250 g), male albino Swiss mice (20–25 g), and male albino guinea pigs (300–350 g) were used in all experiments and were fed *ad libitum*.

Substrates—17-Chloroacetylajmaline[2] (I) and 17-acetylajmaline[2] (II) are odorless, white, microcrystalline powders. They do not have a significant melting point but begin decomposing above 220 and 230°, respectively. Their purity was checked by TLC utilizing silica gel[3] precoated aluminum sheets with a layer thickness of 0.25 mm and F_{254} UV indicator.

The plates were activated at 60° for 1 hr. After the application of about 20 ng of each compound, the plates were developed with

Table I—Distribution of 17-Chloroacetylajmaline, Acetylajmaline, and α-Naphthylacetate Hydrolyzing Esterases in Guinea Pigs

Tissue	Activity[a], μmoles/min/g Protein		
	17-Chloroacetylajmaline	Acetylajmaline	α-Naphthylacetate
Plasma	0.043 ± 0.024	0.044 ± 0.020	0.537 ± 0.029
Liver	1.000 ± 0.029	0.172 ± 0.019	13.698 ± 0.108
Brain	1.080 ± 0.042	0.539 ± 0.023	1.150 ± 0.054
Heart	1.708 ± 0.090	0.449 ± 0.068	2.151 ± 0.102
Leg muscle	0.359 ± 0.017	0.247 ± 0.015	1.855 ± 0.036

[a] Each value represents the mean ± *SE* of the enzymatic activity of three enzyme preparations from different animals.

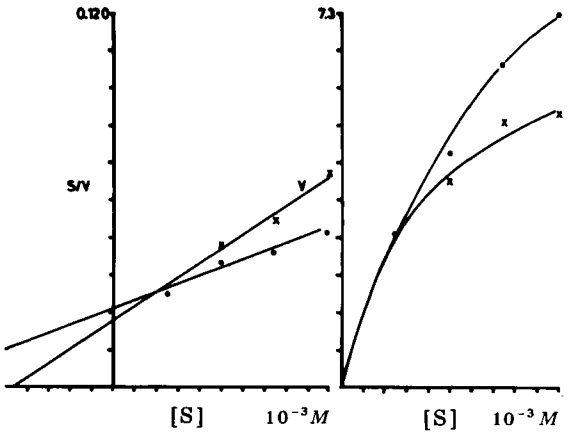

Figure 1—*Woolf plot for the hydrolysis of 17-chloroacetylajmaline in the guinea pig heart. The* K_m *and* V_{max} *values in the atria (×—×) are 4.5 × 10⁻⁴ M and 10 eu, respectively. The* K_m *and* V_{max} *values in the ventricles (●—●) are 9.8 × 10⁻⁴ M and 9.7 eu, respectively. V = initial velocity expressed as micromoles per minute per milligram of protein; S = substrate concentration (molar).*

[1] C. Capra, Laboratorio Farmacologico Inverni & Della Beffa, S.p.A., Milan, Italy, personal communication.

[2] Supplied by Inverni & Della Beffa, S.p.A., Milan, Italy.

[3] Silica gel F_{254}, Merck, Darmstadt, Germany.

Table II—Distribution of 17-Chloroacetylajmaline, Acetylajmaline, and α-Naphthylacetate Hydrolyzing Esterases in Mice

Tissue	Activity[a], μmoles/min/mg Protein		
	17-Chloro-acetylajmaline	Acetylajmaline	α-Naphthyl-acetate
Plasma	0.141 ± 0.050	0.085 ± 0.010	0.354 ± 0.012
Liver	0.818 ± 0.103	0.262 ± 0.051	2.375 ± 0.054
Brain	1.085 ± 0.023	0.087 ± 0.019	1.910 ± 0.070
Heart	0.424 ± 0.090	0.424 ± 0.082	0.169 ± 0.063
Leg muscle	0.066 ± 0.022	0.060 ± 0.020	1.615 ± 0.031

[a] Each figure represents the mean ± *SE* of the enzymatic activity of three enzyme preparations from different animals.

Table III—Distribution of 17-Chloroacetylajmaline, Acetylajmaline, and α-Naphthylacetate Hydrolyzing Esterases in Rats

Tissue	Activity[a], μmoles/min/mg Protein		
	17-Chloro-acetylajmaline	Acetylajmaline	α-Naphthyl-acetate
Plasma	Absent	Absent	0.372 ± 0.010
Liver	0.694 ± 0.141	0.297 ± 0.125	3.766 ± 0.056
Brain	0.556 ± 0.030	0.556 ± 0.028	2.450 ± 0.025
Heart	0.339 ± 0.112	0.339 ± 0.103	0.745 ± 0.093
Leg muscle	0.246 ± 0.020	0.246 ± 0.022	3.033 ± 0.031

[a] Each figure represents the mean ± *SE* of the enzymatic activity of three enzyme preparations from different animals.

methanol. Compounds I and II presented one spot each with R_f values of 0.63 and 0.55, respectively.

α-Naphthylacetate (III) was synthesized as previously described (5).

Preparation of Biological Samples—Tissue and plasma samples were prepared as previously described (5) and were immediately assayed for their enzymatic activity.

Protein Analysis—Protein concentration in plasma and tissues was determined according to Lowry *et al.* (6).

Determination of Esterase Activity—The esterase activity of tissues were determined by a titrimetric method[4] according to Schwark and Ecobichon (7). All substrates were dissolved in 0.05 *M* phosphate buffer at the desired pH value. The volume of the reaction mixture was always 16 ml. A volume of 0.020 ml of appropriate supernate was added to the reaction vessel. The final concentration of the substrates in the vessel was 10^{-3} *M*.

Sodium hydroxide, at a concentration of 0.01 *M*, was used for the titrations, which were carried out at 37° for 6 min. In fact, the reaction was linear for at least 20 min. The pH of the reaction was 7.0 instead of 7.6, which is described to be the optimum for many esterases, because I and II are highly unstable at pH values above 7.2. However, with these experimental conditions in the absence of the enzymatic preparations, the spontaneous hydrolysis of I and II was negligible.

The initial rate of hydrolysis was determined from the titration curves as micromoles of sodium hydroxide utilized per minute per milligram of protein of extract (eu).

RESULTS AND DISCUSSION

Enzyme Distribution in Tissue—To determine the distribution of the enzyme hydrolyzing I and II, a substrate of reference α-naphthylacetate was used; this ester is known to be hydrolyzed, although at a different rate, by all esterases (8–10). Tables I, II, and III show the enzyme distribution in plasma and tissues of the guinea pig, mouse, and rat, respectively.

Compound I was hydrolyzed more rapidly in the heart than in other tissues of the guinea pig, and I was hydrolyzed fourfold faster than II. In rat and mouse hearts, I and II were hydrolyzed at equal rates but this activity was less than in the brain and liver. Since work performed *in vivo* (2) showed that I was much more active than II in the guinea pig only, this finding may suggest that the antiarrhythmic activity is due to the hydrolysis product ajmaline, at the site of action, namely the heart muscle.

It was evident from the high hydrolysis of I in the guinea pig that a large amount of ajmaline was released. Even though ajmaline was reported to be an active compound, if ajmaline itself was administered, it was less effective than 17-chloroacetylajmaline. The reported esterase activity for I present in the heart could have therapeutic significance because it may permit sustained release of ajmaline, the active moiety, at the effected site. It is remarkable that the hydrolysis of 17-chloroacetylajmaline was several fold higher in the guinea pig than in the rat and mouse, in agreement with the more significant pharmacological activity in the former animal species as compared to the two latter species (2).

Kinetic Parameters—Since I acts on the heart, it was of interest to determine the kinetic parameters of the esterase activity in the atria and ventricles of the guinea pig. This species was selected because it showed the highest esterase activity with respect to I among the three species tested.

Figures 1–3 show Woolf plots for I in comparison to II and III in the guinea pig heart. The K_m values in the atria are, for the three substrates, 4.5×10^{-4}, 4.3×10^{-3}, and 5.0×10^{-5} *M*; the V_{max} values are 10.00, 6.14, and 17.09 eu, respectively. In ventricles, the K_m values are calculated to be 9.8×10^{-4}, 7.8×10^{-3}, and 5.0×10^{-5} *M*; the V_{max} values are 9.70, 8.00, and 17.09 eu, respectively.

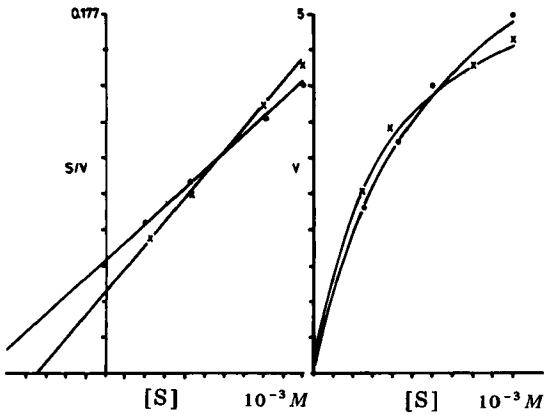

Figure 2—*Woolf plot for the hydrolysis of acetylajmaline in the guinea pig heart. The* K_m *and* V_{max} *values in the atria (×—×) are 4.3 × 10⁻³ M and 6.14 eu, respectively. The* K_m *and* V_{max} *values in the ventricles (●—●) are 7.8 × 10⁻³ M and 8.0 eu, respectively. V = initial velocity expressed as micromoles per minute per milligram of protein; S = substrate concentration (molar).*

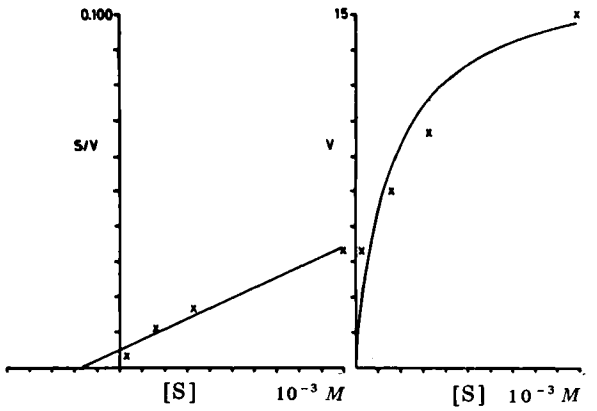

Figure 3—*Woolf plot for the hydrolysis of α-naphthylacetate in the guinea pig heart. The atria and ventricles show the same* K_m *and* V_{max} *values, 5 × 10⁻⁵ M and 17.09 eu, respectively. V = initial velocity expressed as micromoles per minute per milligram of protein; S = substrate concentration (molar).*

[4] pH-Stat, TTT lc Apparatus, Radiometer, Copenhagen, Denmark.

From these results, it is evident that the chloroacetyl ester of ajmaline (I) is more effectively hydrolyzed than the acetyl ester (II) by both the atria and ventricles. Also, the apparent affinity for the enzyme is at least 10 times higher for the chloroacetyl than for the acetyl ester, thus substantiating its *in vivo* effect. Further *in vivo* work is being conducted to determine: *(a)* the levels of ajmaline produced at the site of action after administration of I and II and *(b)* the levels reaching the site of action after administration of ajmaline itself.

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Synthesis and *In Vitro* Evaluation of 8-Hydroxyquinolines as Dental Plaque Inhibitors

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Abstract □ Some 4- and 5-substituted 8-hydroxyquinolines, with predicted log *P* values in the range of 1.48–2.90, were synthesized by modified Skraup reactions or thermal cyclization. These hydroxyquinolines include the 5-methyl, 4,5-dimethyl, 4-methyl, 5-hydroxy-4-methyl, 5-methoxy, 5-methoxy-4-methyl, 4-hydroxy, 4-chloro, 4-amino, and 5-amino analogs. Partition coefficients, antibacterial activity, and antiplaque activity were determined. Four analogs showed *in vitro* antiplaque activity. None of the derivatives with ionizable functions was active.

Keyphrases □ 8-Hydroxyquinoline analogs—synthesis and antibacterial and antiplaque activities □ Antibacterial activity—8-hydroxyquinoline analogs □ Antiplaque activity—8-hydroxyquinoline analogs

If dental plaque is to be controlled, it must be actively removed or its formation must be prevented. Mechanical cleansing is the principal means of removing plaque and its effectiveness is limited. Since dental plaque consists primarily of bacteria and their metabolites, consideration has been given to controlling plaque formation by use of antibacterial agents administered in dentifrices, mouthwashes, troches, or other appropriate vehicles. The present level of knowledge indicates that bacterial plaque is the direct cause of gingivitis and marginal periodontal disease and that without bacterial plaque there will be a great reduction in the occurrence of caries and peridontitis.

Previous research (1) showed that 8-hydroxyquinoline and some of its analogs inhibit *in vitro* plaque formation. It has been suggested that the antibacterial activity of the 8-hydroxyquinolines and their ability to chelate with trace ions on the surface of teeth, with subsequent slow release, should result in good long-term inhibition of dental plaque. Plaque inhibition by 8-hydroxyquinoline has been correlated favorably with the lipid–water partition coefficients. Analogs with log *P* values in the 1–4 range have been shown to have good antiplaque activity.

In an attempt to optimize antiplaque activity and to investigate other physicochemical factors that influence activity in addition to the partition coefficient, the following compounds with calculated log *P* values in the 1–4 range were synthesized and tested: 5-methyl- (I), 4,5-dimethyl- (II), 4-methyl- (III), 5-hydroxy-4-methyl- (IV), 5-methoxy- (VIII), 5-methoxy-4-methyl- (IX), 4-hydroxy- (XIV), 4-chloro- (XV), 4-amino- (XVII), and 5-amino-8-hydroxyquinoline (XVIII).

Compounds I–III, XV, XVII, and XVIII were prepared by standard methods (Table I). Compounds IV, VIII, and IX are unreported derivatives of 8-hydroxyquinoline, while XIV was previously prepared (10) but by a different method than is presented here. Intermediates VI and VII are known compounds (11, 12), but a different method for their preparation is presented in Scheme I.

Antiplaque activity as displayed by 8-hydroxyquinoline requires that a compound be an antibacterial agent. Therefore, the analogs were first evaluated for their *in vitro* antibacterial activity against *Streptococcus mutans* (No. 6715), a pure strain of plaque-forming bacteria. The antiplaque activity of the analogs was evaluated using an *in vitro* screening procedure (2).