Cardiac Arrhythmia Induced by Epinephrine-Aureomycin in Isolated Frog and Turtle Hearts

Paul A. Harvey and Wei Yang

Department of Physiology,
San Francisco State College, San Francisco, California

Much work has been done with the effect of many different substances on cardiac rhythm, although information on the influence of antibiotics on the heart is very meager, despite the hazards involved in their therapeutic use without this knowledge. This fact was our incentive in undertaking the following experiments with aureomycin.[1]

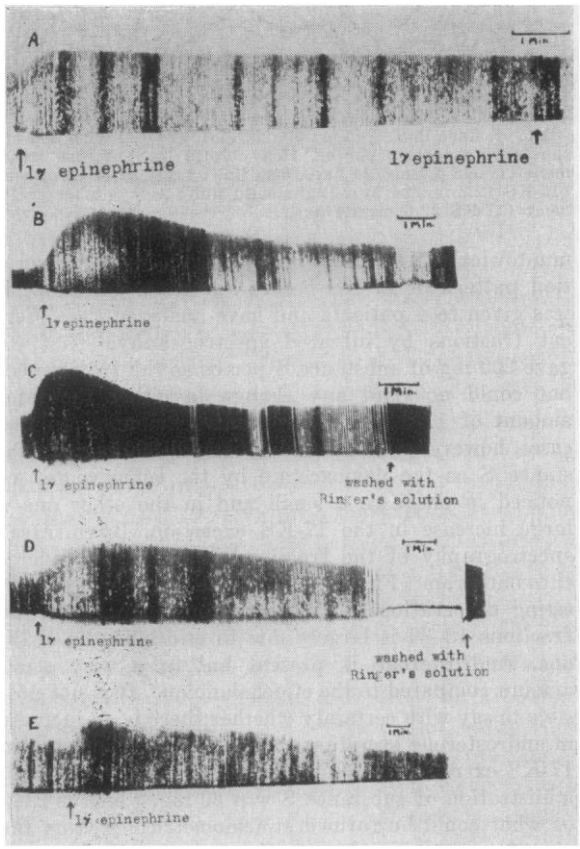

FIG. 1.

Isolated, perfused frog and turtle hearts were used. An animal was pithed, the heart was isolated, cannulated with a Straub cannula and perfused with Ringer's solution. The isolate included usually both vagi for stimulation. A Harvard type inductorium and a Harvard type spring kymograph were used. The emf in the primary circuit was 3 v with a distance of 5 cm between primary coil and secondary coil. A normal tracing was taken following perfusion of the isolate with epinephrine in Ringer's solution, or following epinephrine in Ringer's solution subsequent to perfusion with aureomycin in this same vehicle.

It was found that neither the frog, nor the turtle heart showed irregularities following administration of 1 γ/ml of epinephrine, although both normal chronotropic and inotropic effects were observed (Fig. 1 A). Irregularities began to occur upon addition of 1 γ/ml of epinephrine in a heart that had been treated previously with aureomycin-HCl (1×10^{-4} to 1×10^{-5} g/ml). However, the effect did not begin to appear until 20–80 min following treatment with aureomycin-HCl. They were negatively chronotropic and inotropic in nature and were relatively slight 20 min following treatment with aureomycin, but amounted to complete heart block 80 min following treatment with aureomycin-HCl (Fig. 1 B, C, and D). The degree of arrhythmia was proportional approximately to the length of time that the heart had been treated with aureomycin-HCl. The effects of the perfusates could be abolished following treatment of the isolate with KCl (0.276×10^{-3} to 0.328×10^{-3} g/ml), Fig. 1 E.

Epinephrine-aureomycin induced arrhythmia of the kind observed in isolated, perfused frog and turtle heart preparations shows that aureomycin treatment, prior to epinephrine treatment, first augments and then retards myocardial contractions in frog and turtle hearts. It is unlikely that a contaminant was responsible for the effect, since the aureomycin-HCl used in these experiments was a purified product that was intended to be employed for experimental purposes. Although we have no experimental evidence to account for the phenomenon, some work has been done which suggests that the energy-liberating mechanism that causes myocardial contraction may have been disrupted. Hotchkiss (*1*) suggested that gramicidin may have a bacteriostatic effect at its strength for therapeutic use by preventing normal energy exchange while stimulating a non-phosphorylative type of carbohydrate breakdown. He found that gramicidin increased the rate of respiration in *Staphylococcus aureus,* but inhibited the uptake of inorganic phosphate normally associated with respiration. Loomis (*2*) concluded that aureomycin depressed phosphorylation without inhibiting respiration, and that it resembled gramicidin in this behavior.

Enselberg, Simmons, and Mintz (*3*) found that potassium salts were capable of uniformly reducing or abolishing ventricular extrasystoles, and that bidirectional ventricular and auricular tachycardia could be successfully treated with them. Castledon (*4*) reported that administration of potassium salts resulted in disappearance of extrasystoles, and Sampson and Anderson (*5*) used potassium salts to check auricular and ventricular ectopic beats and tachycardias.

Isolated frog and turtle hearts, perfused with Ringer solutions containing aureomycin, and later perfused with solutions containing epinephrine, developed arrhythmias. When epinephrine was given 20 min after aureomycin, chronotropic and inotropic irregularities appeared about 10 min later, when the pres-

[1] Aureomycin-HCl supplied by the Lederle Laboratories Division of the American Cyanamid Company.

sor effect had almost disappeared. If the time interval was increased to 40 min, the irregularities became more pronounced. When the time interval was 80 min, complete heart block occurred, with failure of ventricular contraction. KCl administration just before perfusion of epinephrine abolished the arrhythmias.

References

1. HOTCHKISS, R. D. *Advances in Enzymol.*, **4**, 153 (1944).
2. LOOMIS, W. F. *Science*, **111**, 474 (1950).
3. ENSELBERG, C. D., SIMMONS, H. G., and MINTZ, A. A. *Am. Heart J.*, **39**, 713 (1950).
4. CASTLEDON, L. I. M. *Brit. Med. J.*, **1**, 7 (1941).
5. SAMPSON, J. J., and ANDERSON, E. M. *J. Am. Med. Assoc.*, **99**, 2257 (1932).

Manuscript received August 28, 1953.

Complementary Enzyme Actions in the Clotting of Milk[1]

R. M. Heinicke

Pineapple Research Institute of Hawaii, Honolulu

According to Berridge (*1*) the clotting of milk by rennin or pepsin is a three-step reaction. The enzyme destabilizes the casein molecule, moderate thermal energy denatures the enzyme-modified molecule, and polyvalent cations crosslink the extended polypeptide chains into a coherent clot. The exact nature of the enzyme action is still obscure.

Below 14° C thermal energy is not sufficient to denature the rennin or pepsin destabilized casein molecule in any convenient time interval. However, if an appropriate protease is present, this denaturation may be brought about enzymatically. For the milk-clotting reaction, this enzymatic denaturing action complements the rennin-like destabilization action.

Many proteolytic enzymes probably can perform both types of enzymatic actions. Examples of this group are pineapple bromelain,[2] papain, and chymotrypsin. Other enzymes at the pH of the milk-clotting test can perform only one of these actions. Thus rennin and pepsin exhibit only the rennin-like destabilizing action at pH 5.3. Trypsin, on the other hand, exhibits principally a protein-splitting action (Table 1). Part of the milk-clotting action of the trypsin sample reported in Tables 1 and 2 may be attributable to chymotrypsin impurities.

If the low temperature milk-clotting ability of pineapple bromelain (Table 2) and papain (*2*) is attributable to two complementary enzyme changes, then it should be possible by combining a rennin-like enzyme with a protein-splitting enzyme to obtain a low-temperature milk-clotting combination. This hypothesis is amply borne out by the data in Table 2. A ratio of 0.44–0.48 milk-clotting units of rennin or pepsin to one protease unit of trypsin gave the best proportions for a low-temperature milk-clotting combination. If a correction is made for the milk-clotting activity of trypsin, the ratio of milk-clotting activity to protease activity is 0.52–0.56.

If similar calculations are made for pineapple bromelain, the ratio of milk-clotting units to protease units is 0.85, quite different from the figure for rennin-trypsin. Furthermore, the addition of either trypsin or rennin to pineapple bromelain increases the unit activity of pineapple bromelain. However, rennin has much more of an activating effect than trypsin.

At the temperature of standard milk-clotting tests, 37.5° C, the thermal denaturation step may proceed more readily than the protein-splitting step (*2*). Hence the standard milk-clotting test would measure principally the rennin-like action of the enzyme. Among different proteolytic enzymes no fixed relation-

TABLE 1

MILK-CLOTTING ACTIVITY AND CASEIN-SOLUBILIZING ACTING OF SEVERAL PROTEOLYTIC ENZYMES AT 37.5° C

Enzyme	Grade	Milk-clotting units*/g	Casein solubilizing units†/g	
			pH 5.3	pH 7.25
Pineapple bromelain	Experimental	4,670	5,500	14,000
Pepsin	N. F.	21,300	Neg.	Neg.
Rennin	N. F. V1	4,270	Neg.	Neg.
Trypsin	N. F.	400	5,000	17,500

* Modified (*2*) Balls and Hoover method (*3*); 5 ml of a 5% milk buffered to pH 5.3.
† Chow-Peticolas method (*4*).

TABLE 2

EFFECT OF COMBINING ENZYMES ON THE TIME NECESSARY TO CLOT 5 ML OF A 5% SKIM MILK SOLUTION AT 4° C

Mg enzyme in 1 ml of added enzyme solution				Minutes for clotting
Bromelain	Trypsin	Rennin	Pepsin	
2.232	—	—	—	12.2
—	4.33	—	—	127.4
—	—	2.24	—	No clot
—	—	—	1.94	No clot
1.116	2.16	—	—	18.0
0.744	2.88	—	—	26.4
0.446	3.46	—	—	35.2
1.116	—	1.12	—	9.3
0.319	—	1.92	—	11.1
0.203	—	2.03	—	19.5
—	3.46	0.45	—	37.1
—	2.88	0.75	—	23.6
—	2.16	1.12	—	17.7
—	1.45	1.49	—	41.2
—	3.99	—	0.15	22.7
—	3.46	—	0.39	16.0
—	3.24	—	0.49	18.0
—	2.88	—	0.64	26.7
—	2.16	—	0.97	30.6

[1] Published with the approval of the Director of the Pineapple Research Institute of Hawaii as Technical Paper No. 214.

[2] For conformity with the rules for naming plant proteolytic enzymes suggested by Greenberg, Winnick, and Lineweaver (*Ann. Rev. Biochem.*, **14**, 31 [1945]) and for the orderly naming of the other enzymes found in the Bromeliaceae, a suggestion has been made to the A.C.S. Committee on Rules, Nomenclature, and Pronunciation that the name pineapple bromelain be substituted for bromelin.