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This Week's Citation Classic®

Entman M L, Levey G S & Epstein S E. Mechanism of action of epinephrine and glucagon on the canine heart: evidence for increase in sarcotubular calcium stores mediated by cyclic 3',5'-AMP. *Circ. Res.* **25**:429-38, 1969.
[Lab. Skeletal Muscle Res., Armed Forces Inst. Pathology, Washington, DC and Cardiology Branch, Natl. Heart Inst., Bethesda, MD]

The findings published in this paper demonstrate that ATP-dependent calcium uptake by a microsomal fraction isolated from heart was stimulated by cyclic-AMP (cAMP) in a concentration-dependent manner. The effect of cAMP was increased by longer preincubations of the microsomal fraction in the presence of ATP. These findings suggested that cAMP-associated agents might modulate cardiac contractility by their effect on sarcoplasmic reticulum calcium flux. [The *SCI*® indicates that this paper has been cited in over 235 publications since 1969.]

Mark L. Entman
Section of Cardiovascular Sciences
Department of Medicine
and
DeBakey Heart Center
Baylor College of Medicine
Houston, TX 77030

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The discovery of cyclic-AMP (cAMP) and its association with various hormones in the 1960s stimulated interest aimed at delineating its role in many different organ systems. In 1965 Robison and coworkers demonstrated that epinephrine's stimulatory effect on the isolated perfused rat heart was associated with elevations in myocardial cAMP concentrations.[1] By the late 1960s the early skeletal muscle studies of microsomal fractions enriched for sarcoplasmic reticulum membranes had been supplemented by studies of cardiac muscle.

The initial studies for this paper, begun at Duke University, were discontinued when I entered the US Air Force and relocated to Washington. I was assigned to the Armed Forces Institute of Pathology where a laboratory was made available to me for these studies. While my laboratory equipment was arriving, I became aware of the work of my coauthors, Levey and Epstein, who were correlating adenylyl cyclase activity and inotropic response. Our collaboration in this work began from our mutual interests.

The paper demonstrated that ATP-dependent calcium uptake by a microsomal preparation made from canine heart was stimulated by the presence of cAMP and that a preincubation with cAMP in the presence of ATP was necessary to demonstrate the stimulatory effects of cAMP. Longer preincubation caused greater stimulation. The mechanism by which cAMP produced this effect on calcium transport and the role of preincubation were unknown at that time. We demonstrated, in another paper published almost concurrently, that the sarcoplasmic reticulum preparation we were using was associated with high levels of adenylyl cyclase activity.[2] This led us to a hypothesis that the sarcoplasmic reticulum might also contain a structured system that regulated its activity and that involved cAMP production and cAMP-induced stimulation of calcium uptake.

In ensuing years, the role of protein phosphorylation in the metabolic effects of cAMP was elucidated. It remained for the classic work of Katz and coworkers to demonstrate the mechanism by which cAMP effected this response.[3] The phosphorylation of phospholamban by a cAMP-dependent protein kinase represented the mechanism by which calcium transport was stimulated. More recently, *in vitro* phosphorylation of phospholamban by calmodulin-dependent protein kinase has been demonstrated,[4] and it exerts a similar stimulatory effect to that seen with cAMP-dependent phosphorylation, although phosphorylation occurs at different sites.

In my opinion, the paper has been highly cited because it represented an initial attempt to relate the cAMP-associated stimulation of cardiac contractility to a specific biochemical step associated with excitation-contraction coupling. The subsequent observations of many laboratories have added much sophistication to these initial observations so that this field continues to grow.

Since we wrote our initial paper, Levey and I collaborated again on a project relating sarcoplasmic reticulum to cAMP-mediated events.[5]

1. **Robison G A, Butcher R W, Oye I, Morgan H E & Sutherland E W.** Effect of epinephrine on adenosine 3',5'-phosphate levels in the isolated perfused rat heart. *Mol. Pharmacol.* **1**:168-77. 1965. (Cited 405 times.)
2. **Entman M L, Levey G S & Epstein S E.** Demonstration of adenyl cyclase activity in canine cardiac sarcoplasmic reticulum. *Biochem. Biophys. Res. Commun.* **35**:728-33. 1969. (Cited 95 times.)
3. **Kirchberger M A, Tada M, Repke D I & Katz A M.** Cyclic adenosine 3',5'-monophosphate-dependent protein kinase stimulation of calcium uptake by canine cardiac microsomes. *J. Mol. Cell. Cardiol.* **4**:673-80. 1972. (Cited 155 times.)
4. **Le Peuch C J, Haiech J & Demaille J G.** Concerted regulation of cardiac sarcoplasmic reticulum calcium transport by cyclic adenosine monophosphate dependent and calcium-calmodulin-dependent phosphorylations. *Biochemistry—USA* **18**:5150-7. 1979.
5. **Entman M L, Bornet E P, Garber A J, Schwartz A, Levey G S, Lehotay D C & Bricker L A.** The cardiac sarcoplasmic reticulum-glycogenolytic complex: a possible effector site for cyclic-AMP. *Biochim. Biophys. Acta* **499**:228-37, 1977. (Cited 10 times.)