PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **147**, 103–105 (1974)

Effect of Acute Cardiac Overload on Intramyocardial Cyclic 3′,5′-AMP: Relation to Prostaglandin Synthesis (38289)

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The heart responds to a chronic pressure or volume overload by increasing its functional mass to meet the work requirements. Extensive research into the mechanisms underlying this adaptive process has unraveled some of the biochemical and cellular changes that accompany cardiac hypertrophy. The very early steps in the initiation of cardiac hypertrophy, however, remain uncertain. Recently, the adenylate cyclase system has been implicated in the regulation of protein synthesis by the acutely overloaded myocardium (1). The present study documents an early increase in intramyocardial cyclic 3′,5′-AMP levels following aortic constriction and provides evidence that this increase is closely related to stimulation of myocardial prostaglandin synthesis.

Methods. Experiments were conducted on adult Wistar rats weighing 250–300 g. Under barbiturate anesthesia, a tracheostomy tube was inserted and connected to a Harvard respirator. The heart was then exposed by a median sternal split and a tie was placed around the thoracic aorta approximately 1 in. from its origin and fastened tightly around an 18-gauge needle which was subsequently removed. Five min later the heart was excised, immersed immediately in an acetone–dry ice mixture, and processed for cyclic 3′,5′-AMP and prostaglandin synthetase activity. A separate group of animals (designated as "controls") were treated identically, the only difference being that the tie around the aorta was left loose. Determinations of cyclic 3′,5′-AMP levels and prostaglandin synthetase activity were made concomitantly with the aortic constriction group.

The effects of indomethacin on prostaglandin biosynthesis and cyclic 3′,5′-AMP levels were tested in both the aortic constriction and the control groups. Indomethacin was dissolved in the drinking water in a concentration of 50 μg/ml and the animals were allowed to drink *ad libitum*; this resulted in an average daily dose of 300 μg indomethacin per kg wt. In the morning of the fourth day, the rats were alloted to either the constricted or the control group.

Cyclic 3′,5′-AMP levels in the myocardium were assayed by the method of Gilman (2) which is based on competition for protein binding of the nucleotide to a cyclic AMP-dependent protein kinase in the presence of a protein kinase inhibitor. The binding reaction was carried out at 10° for 1 hr in a final volume of 200 μl of 20 m*M* potassium buffer containing, in addition to the unknown amount of cyclic 3′,5′-AMP, 14 μg of binding protein, 22.5 μg of protein kinase inhibitor, and ^{3}H-cyclic AMP (0.06 pmole; sp act 2–5 Ci/m*M*). A standard curve was obtained by simultaneous determination with unlabeled cyclic AMP.

Prostaglandin synthetase activity was determined in the hearts of untreated and indomethacin-treated animals by the method of Takeguchi *et al.* (3). This method depends on the detection of a cyclic chromogen derived from PGE_2 in the presence of reduced glutathione (GSH), aromatic compounds as cofactors, and alkali. In the presence of GSH, arachidonic acid is converted preferentially to PGE_2 which, under alkaline conditions, is converted to PGB_2 and is easily detected by a modified Zimmerman reaction.

The effects of PGE_2 and indomethacin on intramyocardial cyclic 3′,5′-AMP levels were determined by the method of LaRaia and Reddy (4). The animals were sacrificed, the hearts quickly removed, and slices of the left ventricle weighing 50–75 mg were prepared with a

Stadie–Riggs tissue slicer. The slices were incubated for 45 min in 2 ml of a modified Krebs–Ringer bicarbonate buffer containing one-half the normal concentration of Ca^{2+}, 1 mg/ml glucose, 1 mg/ml of fat-free bovine albumin, and 1 μCi/ml of ^{14}C-adenine. Incubation was carried out in an atmosphere of O_2-CO_2 (95:5, v/v) at 37° in a metabolic shaker. After this 45-min period, the medium was removed, and fresh medium was added to each flask. After 5 min of temperature equilibration, the agents to be tested were injected into the flask in a volume of 0.1–0.2 ml of buffer with a calibrated tuberculin-type sringe and needle. Following an additional incubation of 5 min, the slices were removed, placed on dry ice–acetone, and processed for cyclic AMP determination as described above.

Results. Intracellular cyclic 3′,5′-AMP levels were significantly higher in the aortic constricted animals: 2.046 pmoles/mg protein compared to 1.421 pmoles/mg protein in the control group ($P < 0.001$). Concomitantly, prostaglandin synthetase activity increased from 2.3 ± 0.01 nmoles PGE_2/min in the sham-operated rats (controls) to 3.9 ± 0.02 nmoles PGE_2/min in the aortic-constricted group ($P < 0.001$). As shown in Table I, both the cyclic AMP rise and prostaglandin synthetase activation were significantly attenuated in the idomethacin-treated animals. The incubation experiments demonstrated an increase in myocardial cyclic 3′,5′-AMP mediated by PGE_2 (Table II). Indomethacin (5 μg/ml) resulted in a small decrease of myocardial cyclic AMP levels but did not have a significant effect on the PGE_2-mediated activation of adenylate cyclase. This decrease in cyclic AMP levels may reflect inhibition of prostaglandin synthetase activation during tissue processing. Indomethacin also inhibited prostaglandin synthetase activity *in vitro*: 0.98 ± 0.01 nmoles PGE_2/min in the presence of 5 μg/ml indomethacin compared to 2.1 ± 0.02 nmoles PGE_2/min in its absence ($P < 0.001$).

Discussion. Sustained increase in cardiac work is associated with augmented rate of protein synthesis and subsequent hypertrophy. The early steps in the sequence of events leading to increased myocardial protein synthesis are not known but several biochemical changes have been described (5–9). An increase in DNA-dependent RNA polymerase occurs as early as 20 min after elevation of aortic perfusion pressure in the isolated working heart (5). This is followed by increased amino acid incorporation into myocardial protein 1 hr after the initiation of the stress (6, 7). Schreiber and his associates have found increased adenylate cyclase activity in the particulate fraction from overloaded left ventricles 10 min after the imposition of the load (1, 8). Cyclic AMP levels at that time were not significantly elevated but this might have reflected prompt destruction of the nucleotide shortly after it initiated its physiologic functions. Stimulation of adenylate cyclase activity has also been described in the hypertrophied heart of the Syrian cardiomyopathic hamster (9).

The possibility that the adenylate cyclase system is involved in the initiation of cardiac hypertrophy is intriguing in view of recent demonstrations that cyclic AMP may be a regulator of RNA transcription (10–12). A direct effect of cyclic AMP on nuclear RNA polymerase has been observed in rat liver (11) and heart (12). The early stimulation of nuclear polymerase activity in cardiac hypertrophy and the increased template activity for RNA polymerase of chromatin isolated from the nuclei of hypertrophied hearts

TABLE I. Effects of Indomethacin on the Changes in Cyclic 3′,5′-AMP Levels and Prostaglandin Synthetase Activity Induced by Aortic Constriction. Numbers in Parentheses Indicate the Number of Animals in Each Group.

		Cyclic 3′,5′-AMP (pmoles/mg. protein)	*P*	PGS activity (nmoles PGE_2/min)	*P*
I.	Sham operated (8)	1.42 ± 0.06		2.3 ± 0.01	
II.	Aortic constricted (8)	2.05 ± 0.08	< 0.001*	3.9 ± 0.02	< 0.001*
III.	Sham operated, indomethacin treated (6)	2.08 ± 0.08	NS**	2.0 ± 0.01	NS
IV.	Aortic constricted, indomethacin treatment (6)	1.07 ± 0.05	< 0.01**	2.4 ± 0.02	< 0.01

* With respect to group I.

** With respect to group II.

TABLE II. The Effect of Indomethacin on the PGE_2-induced Activation of Myocardial Adenylate Cyclase. Numbers in Parentheses Indicate the Number of Animals in Each Group.

	Cyclic 3′,5′-AMP (pmoles/mg. prot.)	*P*
Control (6)	0.412 + 0.08	
PGE_2 (4)	0.690 ± 0.08	<0.01*
Indomethacin (4)	0.381 ± 0.06	NS
PGE_2 and Indomethacin (4)	0.440 ± 0.07	<0.01**

* With respect to control.

** With respect to indomethacin.

(13) indicate that regulation on the transcriptional level is probably fundamental to the initiation of cardiac enlargement.

In the present study, a statistically significant increase in intramyocardial cyclic AMP levels was found as early as 5 min after aortic constriction; this supports the results of the experiments by Schreiber *et al.* (1). One intriguing finding was the possible association of the adenylate cyclase activation to increased prostaglandin synthesis. The stimulation of myocardial prostaglandin synthetase by acute overload is not surprising. Visible dilatation of the cardiac chambers is the first consequence of such overload and might be the responsible stimulus. In other tissues, such as lung and uterus, stretch has been shown to increase prostaglandin synthesis (14). The exact mechanism of the synthetase activation is not known but is related to changes in the cell membrane during stretch.

Stimulation of myocardial adenylate cyclase by prostaglandins has also been found by Klein and Levy (15). The present study demonstrates that this activation can be blocked *in vitro* by indomethacin, a known inhibitor of prostaglandin biosynthesis. Indomethacin was also an effective inhibitor of the overload-induced increase in prostaglandin synthetase activity *in vivo*. Such an effect was less evident in the sham-operated animals. This is in keeping with the concept that prostaglandins are not stored in tissues but are synthesized locally after appropriate stimuli and then rapidly destroyed.

The data of Schreiber *et al.* (1) suggest that the cyclic AMP in acutely overloaded hearts may originate from one of several poorly mixed pools of the nucleotide. Our findings imply that the PGE_2-sensitive adenylate cyclase is the one primarily stimulated during acute cardiac overload since this increase was essentially blocked in the indomethacin-treated animals. This opens the possibility that membrane-bound prostaglandin synthetase may be one regulating step in myocardial protein synthesis.

Summary. Acute cardiac overload induced in Wistar rats by aortic constriction resulted in an early (within 5 min) rise of intramyocardial cyclic 3′,5′-AMP levels and concomitant activation of myocardial prostaglandin synthetase. Inhibition of prostaglandin synthesis by indomethacin pretreatment prevented the increase in cyclic 3′,5′-AMP levels following aortic constriction, suggesting involvement of prostaglandins in the early response of the myocardium to acute overload.

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Received November 12, 1973. P.S.E.B.M., 1974, Vol. 147.