Vol. 81, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1978 Pages 248-253

DISSOCIATION OF cAMP CHANGES AND MYOCARDIAL CONTRACTILITY IN TAURINE PERFUSED RAT HEART

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Received January 11,1978

Summary. It has been determined that the inotropic effects of taurine in the rat heart are not necessarily mediated through changes in cyclic nucleotide levels. Taurine caused a transient 2-fold decrease in cAMP levels, and this was accompanied by a slight (5%), but significant positive inotropic effect. This cannot be explained by an antagonism of cAMP levels by 3', 5' -guanosine monophosphate (cGMP), since the latter remained essentially unchanged. Epinephrine, as is known, caused a rapid increase in cAMP levels. Perfusion with both taurine and epinephrine increased cAMP levels to the same extent as perfusion with epinephrine alone.

Taurine (2-aminoethanesulfonic acid), a naturally occurring amino acid, exerts a wide range of pharmacological effects on the mammalian heart. Of particular interest is the observation that taurine is an effective antiarrhythmic agent against development of epinephrine- or digoxin-induced premature ventricular contractions (1). It was suggested that taurine mediated its effect by preventing the loss of potassium associated with the administration of large does of epinephrine or digoxin (2,3). This hypothesis was based on evidence showing that taurine increased accumulation of potassium in the presence of epinephrine or digoxin (3).

Taurine has also been linked with increased binding and transport of calcium (4-6). Dolara et al. (4) demonstrated that total calcium content is increased in taurine treated guinea pig hearts, and calcium was retained longer during washout experiments. They further showed (5) that the calcium binding rate and content of sarcoplasmic vesicles were increased by taurine. Huxtable

0006-291X/78/0811-0248$01.00/0

et al. (6) have shown that sarcoplasmic reticulum isolated in the presence of taurine had an increased rate of calcium influx.

Taurine has also been seen to have an inotropic effect on the heart (7). Cyclic nucleotides have been implicated in the inotropic effects of catecholamines (8-10) and acetylcholine (9,11) in the heart. Therefore, it was of interest to determine if cyclic nucleotides could be implicated in some of the observed physiological effects of taurine.

Methods and Materials: Hearts from 240-260 gm male Wistar rats were perfused within 45 seconds following decapitation. The standard working heart apparatus used in these studies was a modified version of that described by Neely et al. (12) and used in previous studies (13,14). The coronary system of the heart was perfused from a reservior placed 100 cm above the aortic cannula, while the left atrium received fluid from an atrial reservior maintaned at 13 cm of water. When the left atrial cannula was open, there was a net ejection of fluid against the 100 cm pressure head. Left ventricular pressure was measured with a Statham P23Gb pressure transducer by inserting a 22 gauge needle through the ventricle wall. Pressure work was calculated according to Neely et al. (12).

Two parallel circuits with separate aortic and atrial reservoirs maintained at 37°C were used to permit rapid interconversion of perfusion fluid with a minimum of dead space. The standard perfusate was Krebs-Henseleit buffer (1.25 mM Ca^{++}) supplemented with 5 mM glucose and 10^{-2} units/ml insulin and gased with 95% O_2 giving a resultant pH of 7.4. During a representative experiment, taurine was added to one of the circuits containing the standard perfusate while the other circuit contained the standard buffer without taurine. The physiological and biochemical changes were observed following a rapid transition from standard buffer to the buffer supplemented with 10 mM taurine. In the studies employing epinephrine, the standard perfusate was supplemented only with 15 mM glucose while the variable buffer contained either 0.3 µg/ml epinephrine or epinephrine plus 10 mM taurine. In order to avoid oxidation of epinephrine, the reagent was kept in deionized water until minutes before its use. Hearts were paced with an external stimulator at 320 beats/minute and were allowed to stabilize under pacing for 20 minutes to achieve reproducible steady-state levels of the nucleotides.

At specific time intervals following switch to buffer containing taurine (10 mM), epinephrine (0.3 µg/ml), or both, hearts were rapidly frozen using a Wollenberger clamp precooled in liquid nitrogen. Following lyophilization, a known weight (0.2 to 0.11 gm) of freeze-dried ventricular tissue was homogenized with 2.5 ml of 1% perchloric acid, and 50 µl each of [^{3}H]-cAMP and [^{3}H]-cGMP were added as tracers to determine recovery. Following centrifugation, the supernatant was adjusted to pH 5-6 with 3 M K_2CO_3. The potassium perchlorate precipitate was removed by further centrifugation, and 80-90% of the supernatant was added to an AG 1-X8 anion exchange column in the formate form. After washing with 10 ml of 0.1 M formate, cAMP was eluted off with 10 ml of 2.0 M formate followed by elution of cGMP with 4.0 M formate. These were immediately lyophilized, reconstituted in 1.0 ml of assay buffer, and the percent recovery determined. Cyclic AMP was assayed according to the method of Brown et al. (15), while cGMP was assayed according to Watanabe et al. (16).

Taurine, obtained from Aldrich, was recrystallized from water, and examined chromatographically for purity prior to use. Collaborative Research provided the reagents for the cGMP assay. Bovine adrenals used in the cAMP assay were a gift of Adam Buta, East Texas, Pa. Epinephrine was obtained from Fisher, and

TABLE I

Effects of Taurine Perfusion Upon Cardiac Work and the Tissue Levels of cAMP and CGMP[a]

Experimental Conditions	Time After Addition	Cardiac Work	cAMP	cGMP
	sec	% Control	nmol/g.dry wt.	pmol/g.dry wt.
Control[b]	0	100	5.39±0.42	28.6±1.0
Taurine	15	99.5±0.6	4.18±0.58	
Taurine	30	102.7±1.0	3.21±0.33	24.9±1.4
Taurine	45	105.0±1.0	2.48±0.26	28.7
Taurine	60	106.0±1.0		
Taurine	75	105.0±1.0	5.1	32.9
Taurine	90	105.5±1.0	5.20±0.12	31.6±1.5
Taurine	120	105.0±1.0		
Taurine	150	106.5±1.0		
Taurine	180	107.0±0.4	4.65±0.23	29.5

a. Values shown are means ± S. E. M. of 4-6 hearts.
b. Taurine concentration, 10 mM.

[^{3}H] -cAMP and [^{3}H]-cGMP were obtained from ICN Pharmaceuticals, Inc.

Statistical analysis was performed using the appropriate "t test". Cardiac work was normalized using actually pressure work observed for each heart after 20 minutes of preperfusion before switching to buffer containing taurine. These normalized values were used to determine statistical significance whereas the actual levels of cAMP and cGMP were utilized for statistical analysis. A probability of < 0.01 was used to indicate a significant difference.

RESULTS: Changes in cyclic nucleotide levels in the perfused rat heart were measured as a function of time following rapid conversion from standard Krebs-Henseleit buffer to one containing 10 mM taurine, 0.3 µg/ml epinephrine, or both. Table I shows that perfusion with taurine caused a gradual decrease in cAMP levels by 45 seconds, followed by a more rapid return to control levels. The initial transient decrease in cAMP levels was associated with a 5% increase in cardiac work, while the subsequent increase in cAMP to control levels was associated with no change in contractility. ($p<0.01$) Although there have been many reports linking increased levels of cAMP with increased contractility (8-11), findings of positive inotropic effects accompanied by decreased cAMP levels are rare.

It has been suggested that cGMP mediates the anti-adrenergic effects of acetylcholine by specifically antagonizing the inotropic actions of cAMP (16).

Thus, we also examined for changes in cGMP levels following perfusion with 10 mM taurine. As seen in Table I, taurine appeared to have no effect on cGMP levels. ($p<0.01$)

It was also of interest to determine if the taurine-mediated antagonism of epinephrine-induced premature ventricular contractions was connected to variations in cAMP levels. To test this hypothesis, the cAMP content of hearts treated solely with epinephrine and hearts treated with epinephrine plus taurine were compared. As seen in Table II, there was no significant ($p<0.01$) difference in cAMP levels of the two groups over the time period examined.

Discussion. Our data indicate that taurine mediates a small, but significant, positive inotropic effect in rat hearts perfused with Krebs-Henseleit buffer containing 1.25 mM Ca^{++}. This observed inotropic effect of taurine is inconsistent with the results of Dietrich and Diacono (7), who found taurine to exert a negative inotropic effect in rat hearts. Our study has utilized the more physiological working heart preparation, and the small changes observed appear to be more consistent with *in vivo* studies performed with rabbit, guinea pig, and cat (18).

Numerous studies have attempted to correlate cAMP increases with the positive inotropic effect of catecholamines (8-11). In addition, inhibitors of phosphodiesterase potentiate the catecholamine-mediated increase in the force of contraction (11,19). Not only has it been shown that there is a parallel increase in cAMP levels and the force of contraction after addition of epinephrine to intact heart preparation (8-10), but both of these increases can be prevented by β-adrenergic blockers (20). Recent studies by Watanabe et al. (21) have shown the cAMP increases caused by β-adrenergic stimulation can be greatly attenuated by α-adrenergic agonists. However, taurine's effect does not appear to be mediated by stimulation of the α-adrenergic receptor, since no attenuation of cAMP increase caused by epinephrine was observed. However, not all data supports a link between cAMP and changes in cardiac contractility. Attempts to mimic the positive inotropic effect of catecholamines with addition of cAMP

TABLE II

Effects of Epinephrine and Epinephrine Plus Taurine Upon Tissue cAMP Levels[a]

	nmoles/g.dry wt.		
Time after Addition(sec)	10	20	30
Epinephrine[b]	12.66±1.07	11.89±0.90	7.44±0.31
Epinephrine plus Taurine[c]	13.19±0.98	11.45±1.13	7.05±0.88

a. Values shown are means ± S.E.M. of 4-6 hearts.
b. Epinephrine concentration, 0.3 µg/ml.
c. Epinephrine concentration, 0.3 µg/ml.
Taurine concentration, 10 mM.

to perfused heart preparations have yeilded mixed results (20,22,23), although some success has been achieved with the more-lipid-soluble analogue of cAMP, dibutyryl cAMP (11). Furthermore, it has been reported that catecholamine-stimulated increases in contractile force can be elicited without causing significant changes in cAMP levels or phosphorylase activity (24). Moreover, cAMP levels are not affected by increased contractility due to paired electrical stimulation or increased contraction frequency (25).

Our results do not support the hypothesis that positive inotropic effects are necessarily associated with increases in cAMP levels. Although perfusion with taurine was seen to cause a 2-fold decrease in cAMP levels, this was accompanied by a slight positive inotropic effect. The mechanisms underlying these changes are not clearly established. It is well accepted that taurine mediates changes in calcium flux (3-6), and these changes may be responsible for the augmentation of contraction in taurine-perfused hearts. The decreasing levels of cAMP while contractility is increasing could be due to increased phosphodiesterase activity, which has been shown to be activated by calcium (26). Finally, the "Yin-Yang" theory of Goldberg et al. (27), which views cAMP and cGMP as having antagonistic roles in biological regulation, was also not supported, since cGMP levels remained constant.

ACKNOWLEDGMENT: This work was supported by U. S. Public Health Service Grant HL-18554.

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