Thermal trauma alters myocardial cyclic nucleotides and protein content in mice

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1 This study tested the hypothesis that the systemic effects of burn include altered metabolic activity in the heart. Metabolic activity was studied by measuring alterations in cyclic nucleotide levels and protein concentrations in atrial and ventricular muscle in mice at 14 and 22 days after a 20% body surface area (BSA) burn. Thermal injury was produced on the dorsal surface of anesthetized male CD mice by immersion in water at 95°C for 8 s. This resulted in a full-thickness, 3° scald burn. In atrial and ventricular tissues, levels of adenosine 3':5'-cyclic monophosphate (cyclic AMP) and guanosine 3':5'-cyclic monophosphate (cyclic GMP) were analyzed by ¹²⁵I-radioimmunoassay.

2 The protein content (mg prot g⁻¹ dry wt) increased in the atria. The cyclic AMP content (nmol g⁻¹ dry wt) was significantly increased fourfold and ninefold at 14 and 22 days, respectively, in atria from burned animals compared to controls. The cyclic AMP/cyclic GMP ratios were similarly increased.

3 In the ventricle, the protein content and cyclic AMP levels were not altered, but the cyclic AMP/cyclic GMP ratios (nmol g⁻¹ dry wt) were increased at both 14 and 22 days. These changes both in atria and ventricles were less prominent when cyclic nucleotide concentrations or ratios were expressed as pmol mg⁻¹ protein.

4 The data confirm the hypothesis that a 20% BSA thermal injury evokes effects in sites remote from burn injury such as in the atria and ventricles. These effects include total body weight loss, elevated cyclic AMP, cyclic AMP/cyclic GMP ratios, and protein levels in the atria, and elevated cyclic AMP/cyclic GMP ratios in both atrial and ventricular tissues at 2 and 3 weeks after thermal injury. To prevent underestimation of cyclic nucleotide levels such changes should preferably be expressed on a prot g⁻¹ dry weight basis.

Introduction

Thermal injury to man and animals evokes myocardial dysfunction (Baxter *et al.*, 1966; Aikawa *et al.*, 1978; Martyn *et al.*, 1980). This dysfunction is apparent not only in the acute resuscitation phase but also in the hypermetabolic (recovery) phase of thermal injury (Martyn *et al.*, 1980; 1986). Dysfunction was indicated by an increase in end-diastolic volume and/or decrease in ejection fraction (Martyn *et al.*, 1980; 1981; 1986). This ventricular dysfunction following trauma is thought to increase morbidity and mortality due to the inability of the ventricle to maintain adequate cardiac output (Martyn *et al.*, 1980; Hoffman *et al.*, 1983). The decreased contractility of the myocardium in the hypermetabolic phase is an unexpected finding since circulating catecholamines are elevated in the burned patient (Wilmore *et al.*, 1974). Catecholamines, by altering cyclic nucleotide levels, augment the amplitude and shorten the duration of cardiac contraction (Rasmussen *et al.*, 1972). These events are governed through activation of a variety of protein kinases capable of phosphorylating proteins that regulate Ca²⁺ in the myoplasm. Despite the documented elevations in catecholamines following burn trauma, whether cyclic nucleotides in the myocardium respond appropriately is unknown, although the systemic effects of burn on skeletal muscle cyclic nucleotides have been examined (Tomera & Martyn, 1988; 1989b; Tomera *et al.*, 1988). We have previously reported the effects of infection (*viz.*, endotoxemia) on cyclic nucleotides of the heart when compared to skeletal muscle (Tomera & Martyn, 1990). Infection, a consequence of immunosuppression, is an underlying complication of burn trauma.

In the present study cyclic nucleotides within atrial and ventricular tissues were measured in mice at two and three weeks after a 20% body surface area (BSA) thermal injury.

This was done to test the hypothesis that even a small thermal injury induces changes in cyclic nucleotides, which may account for the inotropic and chronotropic changes observed. Two major findings of the present report were that cyclic AMP/cyclic GMP ratios increase in atria and ventricles and protein levels increase in atria at two and three weeks after thermal trauma. This must be considered when expressing changes in cyclic nucleotides to avoid underestimating the effects of thermal trauma.

Methods

Animals

Male CD mice, 9–10 months, were obtained from Charles River Breeding Laboratories (Wilmington, MA) and maintained on a standard laboratory diet, with water provided *ad libitum*.

Murine thermal injury model

This model was described previously in studies on hypermetabolism, immunosuppression and, more recently, skeletal neuromuscular function (Tomera *et al.*, 1988). All investigations involving mice were conducted in compliance with the guidelines for experimental procedures set forth in the NIH Guide for the Care and Use of Laboratory Animals (Dept. of Health, Education and Welfare No. 80-23, Office of Science and Health Reports, DRR/NIH, Bethesda, MD, U.S.A.). The project was also reviewed and approved by the Animal Studies Committee of the Shriners Burns Institute and the Massachusetts General Hospital. Mice were deeply anesthetized with pentobarbitone (40 mg kg⁻¹ i.p., Lemmon Company, Sellersville, PA, U.S.A.) prior to shaving; anaesthesia was considered adequate when the mice did not show a reflex withdrawal response when their toes were compressed

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with haemostats. The anaesthetized mice were shaved in an area corresponding to 20% BSA, calculated for individual mice according to the equation $A = K(Wt)^{2/3}$, where A = area, Wt = weight, and K = proportionality constant (12.3 for mice), (Schildt & Nilsson, 1970). This predefined BSA was then immersed in 95°C water for 8 s and immediately removed. No mortality using this size of BSA injury was encountered. Control mice did not suffer thermal injury but were prepared in a similar fashion and immersed in lukewarm water.

Subsequent to scalding, a full-thickness 3° burn injury having sharp margins resulted. While recovering from the anaesthesia and trauma animals were kept warm in an ambient environmental temperature of 26°C. All animals were resuscitated with 0.9% saline (4 mg kg⁻¹/° burn, i.p.) and received no additional anaesthesia. The burn wound was treated with applications of silver sulphadiazine (1%, Silvadene Creme, Marion Laboratories, St. Louis, MO, U.S.A.), and gauze dressings were applied. After 4 h the animals were returned to their cages and given 0.9% saline as drinking water with food *ad libitum* for 1 week after burn injury. After this first week they were returned to normal tap water. No antibiotics were administered since burn injury including resulting complications was the aim of the study.

Protein content

Replicate heart tissues, paired to those used for cyclic nucleotide determinations, were used to assay changes in total protein content. Tissue digests were made in 1 M NaOH (Lowry *et al.*, 1951). Actual samples were derived from mice killed on Days 14 and 22. Protein content was expressed by the following equation:

$$\underbrace{\text{total protein}}_{\text{mg prot g}^{-1} \text{ dry wt.}} = \underbrace{\text{Lowry assay}}_{\text{mg prot g}^{-1} \text{ wet wt.}} \times \underbrace{\text{wet/dry wt. ratio}}_{\text{g wet g}^{-1} \text{ dry wt.}}$$

Cyclic nucleotide determinations

Hearts were excised from mice that were killed by cervical dislocation. Left ventricular and atrial tissues were rinsed free of blood in a cold sodium acetate buffer (0.05 M, pH 6.2), then immediately frozen in liquid nitrogen and stored at -70°C. Levels of adenosine 3':5'-cyclic monophosphate (cyclic AMP) and guanosine 3':5'-cyclic monophosphate (cyclic GMP) in heart muscle were measured with ¹²⁵I-radioimmunoassay (RIA) kits purchased from New England Nuclear Corporation (Boston, MA, U.S.A.). On the day of the assay, the frozen muscles were thawed in trichloroacetic acid (6% TCA) and homogenized to a tissue slurry. All samples were analyzed by RIA in duplicate (Steiner *et al.*, 1972).

Cyclic nucleotide levels were expressed in terms of pmol mg⁻¹ prot. To account for any change in myocardial protein, they were alternatively expressed on a nmol g⁻¹ dry wt. basis by the following equation:

$$\underbrace{\text{total cyclic AMP}}_{\text{nmol g}^{-1} \text{ dry wt.}} = \underbrace{\text{cyclic AMP by RIA}}_{\text{nmol mg}^{-1} \text{ prot}} \times \underbrace{\text{Lowry assay}}_{\text{mg prot g}^{-1} \text{ wet wt.}} \times \underbrace{\text{wet/dry st. ratio}}_{\text{g wet/g dry wt.}}$$

where nmol g⁻¹ dry wt. is the product of several procedures performed in duplicate on tissue from the same animal. ¹²⁵I-RIA was performed on the supernatant, and the mg prot was measured on tissue pellets of previously homogenized and centrifuged samples to obtain nmol mg⁻¹ prot, mg prot g⁻¹ wet wt. was calculated from samples previously weighed and digested for Lowry determination and from wet/dry wt. ratios. Actual sample sizes ranged between 100–300 mg wet wt.

Total body weights

All mice were weighed throughout this study at the various time intervals defined within this paper.

Statistical analyses

All values depict mean ± s.e.mean. Statistical significance was determined from treatment of the data by analysis of variance (ANOVA). Comparisons were made for each of the thermally injured groups relative to time-matched controls.

Results

Total body weight changes

Changes in total body weight loss in response to the 20% BSA burn are shown in Table 1 for Days 14 and 22 post-burn. All burned animals showed a weight loss that was significantly greater than their time-matched controls. This weight loss ranged between 6–9% of total body weight. It should be noted that control mice of this age do not typically gain weight at 10 months (Tomera & Martyn, 1989a).

Atria

Muscle protein and cyclic nucleotide changes The myocardial tissues in each group were assayed for total protein content in pre-weighed wet wt. tissue samples, and the tissue wet/dry wt. ratios were also determined. The wet/dry wt. ratios for both the control and the thermally-injured groups did not differ significantly in either the atria or the ventricles. Therefore, the wet/dry wt. ratios for the control and thermally-injured groups were 4 ± 1 ($n = 47$, in atria $n = 5$ for each group of controls and burned mice at Days 14 and 22; and in ventricles at Day 14, controls = 8 and burned mice = 7, at Day 22 controls = 6 and burned mice = 6). This ratio was used in estimating cyclic nucleotides on a nmol g⁻¹ dry wt. basis. Figure 1 shows a threefold increase in protein (mg protein g⁻¹ dry wt.) in the atria at Days 14 ($P < 0.05$) and 22 ($P < 0.001$), respectively, as a consequence of thermal injury. The cyclic AMP changes expressed as pmol mg⁻¹ protein at 14 and 22 days after burn and in controls are shown in Figure 2. At 22 days after thermal injury, a significant increase in cyclic AMP content in the atria was noted; no changes were observed at 14 days. When the cyclic AMP data were re-expressed as nmol g⁻¹ dry wt. to take into account the burn-induced increases in atrial protein concentrations, significant increases in cyclic AMP content were noted at both 14 and 22 days after burn. A disparity in the attainment of significant changes in cyclic AMP levels existed when expressed as pmol mg⁻¹ prot versus nmol g⁻¹ dry wt. At Day 14 after thermal injury, a significant fourfold increase ($P < 0.05$) in cyclic AMP levels (nmol g⁻¹ dry wt.) was observed, while if expressed alternatively as pmol g⁻¹ prot, no change was discerned. At Day 22 a ninefold increase ($P < 0.001$) in cyclic AMP was observed when expressed as nmol g⁻¹ dry wt., compared to a twofold increase ($P < 0.001$) when expressed as pmol mg⁻¹ prot, which did not consider protein increases. Thus, these results show greater differences in the atrial cyclic AMP levels of

Table 1 Effect of burn injury on total body weight (g)†

Groups (n)	Postburn days	
	Day 14	Day 22
Controls (5)	44.26 ± 0.39	44.92 ± 0.31
20% BSA burn (n = 5)	40.82 ± 0.38*	41.86 ± 0.87*

Values are mean ± s.e.mean.

* $P < 0.01$, ANOVA.

† Time-matched controls.

Figure 1 Increased protein levels within the atria at Days 14 and 22 after thermal injury. Stippled columns, control; solid columns, burn. Columns show mean values in terms of mg prot g⁻¹ dry wt.; (number of observations), s.e.mean shown by vertical bars. **P* < 0.05, ***P* < 0.025.

thermally injured mice at both Days 14 and 22 when converted to a nmol g⁻¹ dry wt. basis. The cause and importance of these differences is discussed in the following section. No significant changes were observed in cyclic GMP levels when assessed by either method (Table 2).

Figure 2 Increasing atrial cyclic AMP levels at Days 14 (nmol g⁻¹ dry wt.) and 22 (pmol mg⁻¹ prot, nmol g⁻¹ dry wt.) after thermal injury. Stippled columns, pmol mg⁻¹ prot; hatched columns, nmol g⁻¹ dry wt. Columns show mean values expressed by both methods (number of observations); vertical bars show s.e.mean. Day 14 levels were not increased when expressed as pmol mg⁻¹ prot, **P* < 0.05, ***P* < 0.001.

Relationships of atrial protein to total body weight Because there was at least a threefold increase in atrial protein between the second and third week after burn, these data were tested for any form of systemic effect that might prove responsible. When atrial protein (mg prot g⁻¹ dry wt.) data were correlated to total body weight, a non-linear relationship was observed (i.e. $y = 125x^2 - 10890x + 238127$, $R^2 = 0.79$, $n = 15$). This relationship indicated that as total body weight loss occurred, levels of protein content within the atria increased.

Cyclic AMP/cyclic GMP ratios Figure 3 shows the expression of cyclic AMP/cyclic GMP in the atria by both previously discussed methods. At Day 14 the cyclic AMP/cyclic GMP ratio was increased threefold (*P* < 0.05, ANOVA two-way) in the thermally injured group when protein changes were considered (i.e., when expressed on a nmol g⁻¹ dry wt. basis). No change was observed when expressed in pmol mg⁻¹ prot; this observation confirms the importance of accounting for protein changes when changes in cyclic nucleotides are considered. However, at Day 22 the ratios expressed by both methods had increased equally, by twofold (*P* < 0.001).

Relationship of atrial cyclic AMP/cyclic GMP ratios to total body weight To examine the effect of total body weight loss on atrial cyclic nucleotide ratios, a non-linear regression analysis was performed. A relationship ($y = 80.31x^2 - 2678x + 59405$, $R^2 = 0.50$, $n = 20$) indicated that as total body weight decreased, the cyclic AMP/cyclic GMP ratio increased. This relationship was only observed when the data of the cyclic AMP/cyclic GMP ratio were derived from nucleotide concentrations expressed as nmol g⁻¹ dry wt.

Ventricles

Muscle protein No significant changes in ventricular protein levels were detected at two and three weeks after thermal

Figure 3 Increased atrial cyclic AMP/cyclic GMP ratios at Days 14 (nmol g⁻¹ dry wt.) and 22 (pmol mg⁻¹ prot, and nmol g⁻¹ dry wt.) after thermal injury. Stippled columns, pmol mg⁻¹ prot; hatched columns nmol g⁻¹ dry wt. Columns show mean values expressed by both methods (number of observations); vertical bars show s.e.mean. **P* < 0.001.

Table 2 Myocardial cyclic GMP levels at 2 and 3 weeks after thermal injury

Tissues	Time after thermal injury			
	Day 14		Day 21	
	Control	Thermally Injured	Control	Thermally Injured
Atria				
pmol mg ⁻¹ prot	0.030 ± 0.011 (5)	0.081 ± 0.051 (5)	0.032 ± 0.011 (5)	0.022 ± 0.001 (5)
nmol g ⁻¹ dry wt.	0.009 ± 0.005 (5)	0.003 ± 0.002 (5)	0.031 ± 0.030 (5)	0.070 ± 0.002 (5)
Ventricle				
pmol mg ⁻¹ prot	0.153 ± 0.071 (8)	0.012 ± 0.004 (7)	0.332 ± 0.181 (6)	0.012 ± 0.041 (6)
nmol g ⁻¹ dry wt.	0.013 ± 0.006 (8)	0.022 ± 0.011 (7)	0.042 ± 0.032 (6)	0.031 ± 0.012 (6)

Values are mean ± s.e.mean (number of observations).

Figure 4 Ventricular cyclic AMP levels were not significantly different at Days 14 and 22 after thermal injury. Stippled columns, pmol mg^{-1} prot; hatched columns, nmol g^{-1} dry wt. Columns show mean values (number of observations) with s.e.mean shown by vertical bars.

injury. The values (mg prot g^{-1} dry wt.) obtained were: Day 14 ($n = 7-8$), control 150 ± 50 , burn 150 ± 60 ; Day 22 ($n = 6$), control 140 ± 45 , burn 230 ± 50 .

Cyclic AMP and cyclic GMP Unlike atrial tissues, ventricular tissues did not show any significant change in either cyclic AMP (Figure 4) or cyclic GMP (Table 2) levels at two and three weeks after thermal injury when assessed by either method.

Cyclic AMP/cyclic GMP ratios These data, when expressed by either method, showed significant elevations in cyclic AMP/cyclic GMP ratios (Figure 5). In ventricular tissues a 17 fold ($P < 0.05$) increase was observed in the ratio at Day 14 when depicted either as pmol mg^{-1} prot or as nmol g^{-1} dry wt. By Day 22, the ventricular cyclic AMP/cyclic GMP ratio was still increased by eight to 11 fold. These observations indicate a pronounced systemic effect at this time on the ventricle as compared to the atria (twofold, Figure 3).

Discussion

This study evaluated the effects of thermal trauma on both atrial and ventricular tissues. In each tissue cyclic AMP and cyclic GMP levels were measured and expressed in two different ways: the first expressed both cyclic AMP and cyclic GMP in terms of pmol mg^{-1} prot; the second considered increases in myocardial protein levels, which ultimately led to the expression of the data on a nmol g^{-1} dry wt. basis. Cyclic AMP/cyclic GMP ratios were determined from each of these forms of data expression. In atria the individual cyclic AMP

Figure 5 Increased ventricular cyclic AMP/cyclic GMP ratios at Days 14 and 22 after thermal injury. Stippled columns, pmol mg^{-1} prot; hatched columns, nmol g^{-1} dry wt. Columns show mean values expressed by both methods (number of observations); vertical bars show s.e.mean. * $P < 0.05$, ** $P < 0.025$.

values determined by each method of expression varied if corrections for protein increases were implemented. The cyclic AMP/cyclic GMP ratios in atria and ventricles at Day 14 and 22 were found to be greatly increased when protein was considered. In the ventricle the cyclic AMP and cyclic GMP changes were not changed by burn trauma when expressed by either method. The cyclic AMP/cyclic GMP ratios, however, were increased when expressed by either method. This suggests the implementation of the following considerations when evaluating myocardial pathophysiology resulting from thermal trauma: (1) typical concentrations of substrates, e.g. pmol cyclic AMP , when expressed on a mg prot basis, may be underestimated or overestimated depending on the direction of the change in protein content; (2) to prevent this, protein changes (mg prot g^{-1} dry wt.) should be considered and used to express the substrate on a nmol g^{-1} dry wt. basis; and (3) ratios of cyclic AMP/cyclic GMP may be significantly increased as a result of decreases in the denominator along with commensurate increases in the numerator even though the individual changes may not in themselves be significantly different.

Although all ratios could be expected to be equal when based on cyclic nucleotide levels expressed as pmol mg^{-1} prot and/or pmol g^{-1} dry wt, this was not the case, as shown in Figure 3. The ratios cyclic AMP/cyclic GMP (Figures 3 and 5) differed when expressed per mg of protein and per g of dry weight. The absolute value of the individual nucleotide changed with each form of expression. Although the ratio was expected to remain the same, since both cyclic AMP and cyclic GMP pmol mg^{-1} prot were multiplied by the same factors (i.e. mg prot/wet wt. and $\text{wet/dry weight ratio}$, see Methods, *Cyclic nucleotide determinations*), it did not. This was thought to be due to the variation of data within the burn groups and the controls. This can be appreciated in observing cyclic GMP values (Table 2, cyclic GMP values (pmol mg^{-1} protein) in ventricle 0.332 ± 0.181 in controls on Day 21 and 0.153 ± 0.71 in another control group on Day 14). Despite such variations, no difference of cyclic AMP/cyclic GMP ratios was observed. Variations in atrial cyclic GMP were even greater in atria from the burn groups at Day 14 (i.e. Table 2, 0.081 ± 0.051 pmol mg^{-1} prot) and may account for the difference in cyclic AMP/cyclic GMP ratios within the burn groups. These results demonstrate that a careful evaluation of data within the circumstances of burn trauma is required.

The cyclic AMP/cyclic GMP changes (2–2.5 fold) are comparable to the effects of β -adrenoceptor agonists such as isoprenaline (10^{-6} M) on cyclic AMP in intact embryonic chick hearts (Linden, 1987). Although the fold changes may not be as great as those achieved with alternative agonists they were comparable to other studies. An explanation for a reduced change would include desensitization of adenylate cyclase by catecholamines (Aprille *et al.*, 1979). No studies of the effects of isoprenaline on atria and ventricles from burned animals have yet been performed.

Thus, the validity of the evaluation of burn-induced changes in biochemical parameters should be based on a nmol g^{-1} dry wt. basis to circumvent artifactual estimates. This is further attested to by control values (pmol mg^{-1} prot or nmol g^{-1} dry wt.), which fall within acceptable limits in spite of various methods currently available for tissue sampling. No mortality was encountered at burn injury to 20% BSA. However, this model shows that even small BSA dermal burns can provoke changes in other organ systems (e.g. heart) by burn-induced mediators that are systematically circulated throughout the body by the blood. Therefore, such organ systems, although distant from the focal area of burn injury, are still subjected to a trauma response, even for small BSA burns (i.e. 20%).

An increase in atrial protein concentrations was observed in burned animals despite a decrease in total body weight. It has been previously observed that there is also an increase in protein concentrations in skeletal muscle following burn trauma despite total body weight loss (Tomera & Martyn,

1988). Although total body protein turnover rates are increased following burn trauma (Shangraw & Turinsky, 1982), it is unclear why there is an increase in protein concentrations in the atria. This finding is not peculiar to burn trauma. In protein-calorie malnutrition, where the weight loss is mechanistically different from burn trauma (Shephard *et al.*, 1986), it has also been noted that a decrease in body weight is associated with an increase in atrial and ventricular weight when normalized to body weight (Benfey *et al.*, 1983). The fact that there was no change in wet/dry weight ratios in either atrial or ventricular tissue requires additional clarification. Although the wet dry weight ratios were unchanged, protein concentration increased substantially in the atria (Figure 1). It would appear reasonable that when protein concentration increases, the wet/dry weight ratio would decrease provided there was no oedema. Decreased wet/dry weight ratios were not observed. Therefore, it would appear that oedema in the wet tissues may prevent the wet/dry weight ratios from changing.

In the present study no changes in ventricular protein or cyclic AMP concentrations were observed. This disparate response between atrial and ventricular muscle with respect to protein and cyclic AMP concentrations is difficult to explain. Although no functional studies were performed, it is tempting to conclude that burn-induced ventricular dysfunction documented previously (Baxter *et al.*, 1966; Aikawa *et al.*, 1978; Martyn *et al.*, 1980; 1986) may be related to the inability of the ventricle to hypertrophy (to increase in protein concentration) and increase contractility (via increased cyclic AMP levels) in response to chronic hypermetabolic demands. The lack of increase in cyclic AMP levels in ventricular muscle is surprising since catecholamines (Wilmore *et al.*, 1974), which increase cyclic AMP levels via β -adrenoceptors, are increased in burn trauma. This finding, however, is consistent with the findings in endotoxemia, where despite elevations of catecholamines, no increases in myocardial cyclic AMP levels are evident (Shephard *et al.*, 1986).

Inotropy in the ventricle is thought to be mediated via elevations of cyclic AMP levels (Entman, 1974). Despite a burn-induced increase in O_2 consumption (Tomera *et al.*, 1988) and an increased catecholamine secretion (Wilmore *et al.*, 1974; Aprille *et al.*, 1979), cyclic AMP levels in ventricular muscle were not elevated. Hearts of thermally injured patients may be working on the brink of insufficiency because of their inability to increase cyclic AMP levels. This inadequacy may be characteristic of the systemic effects of thermal injury on the heart despite the high plasma catecholamine levels in this state documented by others (Wilmore *et al.*, 1974; Aprille *et al.*, 1979). Sustained inotropy through β -adrenoceptor stimulation

is difficult because of intense and repeated exposure to endogenous β -agonists, which can diminish both receptor population (down regulation) and agonist affinity (desensitization) (Motulsky & Insel, 1982). In this circumstance, use of exogenous agents that increase cyclic AMP via β -receptors would be futile.

It is interesting to note that by three weeks after thermal injury the eight to 11 fold increase in ventricular cyclic AMP/cyclic GMP ratios has declined from the 17 fold maxima attained at two weeks. This observation suggests that sympathetic compensatory mechanisms may be waning at three weeks. It further provides biochemical evidence (e.g. elevated cyclic AMP/cyclic GMP ratios) to support the previous observation (Hilton *et al.*, 1986) that direct myocardial depression did not occur. The overall significance of elevated cyclic AMP/cyclic GMP ratios as phosphorylation indices within the cardiovascular system has been previously established for other disease states (Amer, 1975). However, in light of the disparities in data obtained within cyclic AMP levels (Figure 2) when expressed on either a pmol mg^{-1} prot or a nmol g^{-1} dry wt. basis, it is difficult to say which, if any, of the parameters (cyclic AMP, cyclic GMP, cyclic AMP/cyclic GMP) is the most important index in monitoring myocardial performance as a consequence of thermal injury. Recent studies, have shown that the relationship between myocardial metabolism, in particular the myocardial status of energy-rich substrates and phosphates and myocardial function, is not as clearly defined as had been thought (Isselhard, 1988).

In future studies of thermal injury it would be beneficial to examine serially the relationships between cyclic nucleotides and their ratios with other factors (e.g. cardiac function, metabolism, and haemodynamics). Measurements of high-energy phosphate changes monitored by nuclear magnetic resonance (n.m.r.) and correlated to functional changes are planned. Applications of this work could utilize the suggestion of others (Schaub & Kunz, 1986) to combine cardiac glycosides with β -antagonists. The glycosides would (1) promote the availability of Ca^{2+} for contraction; (2) decrease phosphodiesterase interaction by Ca^{2+} -calmodulin interactions (Potter *et al.*, 1980); and (3) maintain inotropism through a mechanism not affected by β -antagonists.

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