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Protective Action of Methylprednisolone on the Isolated Perfused Rat Heart Following Severe Hypoxia[1,2] (40101)

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In 1953, Johnson and coworkers (1) suggested that cortisone treatment limited the extent of myocardial damage after myocardial infarction. In their study these investigators found that low doses of cortisone diminished the size of experimental myocardial infarction. Since this early report, there have been conflicting reports regarding the efficacy of corticosteroids in attenuating the deleterious effects of myocardial ischemia (2). More recent studies (3, 4) have indicated that treatment of animals with steroids can limit the size of a myocardial infarction induced by experimental coronary occlusion. Ehbid *et al.* found that large doses of hydrocortisone given intravenously caused regression of abnormal S-T segment elevations of the electrocardiogram both in experimental myocardial infarction in dogs and in recent myocardial infarction in humans (5). More recently Libby *et al.* (3, 7) have studied the effect of large doses of hydrocortisone on the size of experimental myocardial infarction. By separate measurements of electrocardiographic, enzymatic and histologic criteria, these investigators found that corticosteroids decreased myocardial ischemic injury. Furthermore, it was also found that pharmacologic doses of hydrocortisone helped prevent subsequent necrosis even when given as late as 6 hr after the experimental coronary artery occlusion. These studies, although determining myocardial injury by monitoring cardiac enzyme activity and electrocardiographic changes which occur after a completed infarction, have not addressed themselves to evaluating the potential benefit of corticosteroids in enhancing the functional performance of the heart after an hypoxic or ischemic insult. However, Busuttil and coworkers, using an intact canine preparation, demonstrated that hearts of methylprednisolone pretreated dogs exhibited significantly better cardiac performance after aortic conclusion than did controls (6). The question still remained concerning the salutory effect of steroids on hearts that have been exposed to hypoxia. To this end, the present study was designed to determine whether pretreatment with a pharmacologic dose of the glucocorticoid, methylprednisolone, affords protection to the hypoxic isolated perfused rat heart and enhances the recovery of the heart during a period of reoxygenation.

Methods and materials. In this study hearts were obtained from male Sprague-Dawley rats (250–300 g) pretreated (ip) with either methylprednisolone sodium succinate 30 mg/kg 18 hr and 1 hr prior to sacrifice or the vehicle of methylprednisolone consisting of benzyl alcohol in 0.9% NaCl. The methylprednisolone sodium succinate was a generous gift of the Upjohn Company.

Coronary perfusion was conducted with an Anderson perfusion apparatus that contained modified Tyrode's solution equilibrated with 95% O_2 and 5% CO_2 (pO_2 450–500 mm Hg) and maintained at 37° and pH 7.4 ± 0.05. The ionic composition of the Tyrode's solution in millimoles per liter was NaCl, 120; KCl, 5; $CaCl_2$, 4; $MgSO_4$, 0.6; $NaHCO_3$, 30; KH_2PO_4, 0.6 and glucose 5.0. An hypoxic perfusion medium (pO_2 10–20 mm Hg, pH 7.4 ± 0.05) was produced by gassing an identical Tyrode's solution with 95% N_2 and 5% CO_2 for 30 min. Both oxygenated and hypoxic perfusion media were set in a parallel system so that with the turn of a stopcock, either medium could be used for coronary perfusion. In order to determine the effects of the lack of an exogenous energy substrate on cardiac performance, one series of vehicle-treated and steroid-treated hearts was perfused with glucose-free Tyrode's.

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In all experiments, perfusion pressure was maintained constant at approximately 55 mm Hg. A calibrated Grass Ft 0.03 force displacement transducer, connected to the heart by a silk suture attached to a hook placed into the cardiac apex, was used to measure force of contraction. This transducer was mounted on a movable assembly so that sufficient tension could be placed on each heart to place the resultant contraction at the peak of its length-tension curve. Contraction was recorded as grams of developed tension on a Grass model 7 polygraph. Heart rate was maintained constant throughout the experiment by constant pacing at a rate 20% above the intrinsic rate by square wave stimuli (4-8V, 4 msec duration) delivered from an E and M stimulator.

Perfusion of rat hearts was conducted in the following manner. Hearts in vehicle-pretreated and methylprednisolone-pretreated groups were equilibrated for a period of 12–15 min with oxygenated Tyrode's solution (pH 7.4, 37°), then perfused for 3 or 5 min with hypoxic medium, and finally reperfused with oxygenated Tyrode's for 5 or 10 min to determine recovery from hypoxia. Hearts perfused with glucose-free Tyrode's solution were exposed to hypoxic medium for 3 min and recovered for 5 min and hearts perfused with Tyrode's solution containing 5 m*M* glucose were exposed to hypoxia for 5 min and recovered for 10 min.

These periods of hypoxic exposure were selected after it had been first determined that an hypoxic insult of this duration would depress myocardial function to approximately 10% of control. Longer periods of hypoxia usually precluded recovery while shorter periods did not provide a sufficient insult to consistently evaluate recovery.

Results. Table I summarizes the effects of hypoxia on force of contraction of the isolated rat heart perfused with glucose containing Tyrode's solution. There is no significant difference in force of contraction between hearts of vehicle-treated and methylprednisolone-treated animals. Within 1 min of hypoxic perfusion there is a significant reduction in the force of contraction (grams of developed tension) of hearts of both vehicle-treated and methylprednisolone-treated rats. This decline in developed tension progresses in both groups of hearts to a value of 2.2 g and 2.9 g in the vehicle-treated and methylprednisolone-treated groups, respectively, at 5 min of hypoxic perfusion. This represents an approximate 90% reduction in force of contraction in both of these groups.

Also shown in Table I is the force of contraction produced by the hearts during the period of recovery (reoxygenation). Throughout the reoxygenation period hearts from methylprednisolone-treated rats develop more tension than do hearts from vehicle-treated animals. Moreover, the force of contraction is significantly greater ($P < 0.05$) in the former group at 3, 5, 7 and 10 min of reoxygenation. By 10 min of reoxygenation the methylprednisolone-treated group develops 22.2 ± 1.1 g of tension (approximately 100% of the prehypoxic value) while the vehicle-treated group only develops 10.5 ± 0.84 g of tension (approximately 54% of the prehypoxic value) at this same time period.

In Table II is shown the effects of hypoxia on the force of contraction of the isolated rat heart perfused with glucose-free Tyrode's solution. The fall in developed tension during hypoxia is qualitatively and quantitatively similar in both groups of hearts in this series of experiments. By 3 min of hypoxic perfusion, hearts from vehicle-treated and methylprednisolone-treated groups develop 0.63 ± 0.13 and 1.0 ± 0.43 g of tension, respec-

TABLE I. EFFECT OF METHYLPREDNISOLONE AND HYPOXIA ON FORCE OF CONTRACTION IN ISOLATED RAT HEARTS PERFUSED WITH TYRODE'S SOLUTION CONTAINING GLUCOSE.[a]

	Contractile force (grams tension)	
Time periods	Vehicle-treated	Methylprednisolone-treated
Prior to Hypoxia	19.6 ± 1.2	22.8 ± 1.3
Hypoxia		
1 min	7.3 ± 0.81	9.3 ± 0.63
3 min	3.3 ± 0.44	4.6 ± 0.60
5 min	2.2 ± 0.26	2.9 ± 0.31
Reoxygenation		
1 min	6.1 ± 1.1	10.4 ± 0.91[b]
3 min	11.4 ± 1.4	18.3 ± 0.77[b]
5 min	12.3 ± 1.8	22.1 ± 1.2[b]
7 min	11.5 ± 1.6	23.4 ± 1.0[b]
10 min	10.5 ± 0.84	22.2 ± 1.1[b]

[a] 5 m*M* glucose added to Tyrode's perfusion medium. Each value represents the mean ± SEM; $n = 7$ for steroid-treated group; $n = 7$ for vehicle-treated group.

[b] Indicates significant difference ($P < 0.05$) when methylprednisolone-treated group is compared to vehicle-treated group.

TABLE II. EFFECT OF METHYLPREDNISOLONE AND HYPOXIA ON FORCE OF CONTRACTION IN ISOLATED RAT HEARTS PERFUSED WITH GLUCOSE-FREE TYRODE'S SOLUTION.[a]

Time periods	Contractile force (grams tension)	
	Vehicle-treated	Methylprednisolone-treated
Prior to Hypoxia	14.4 ± 0.85	15.0 ± 1.6
Hypoxia		
1min	3.6 ± 0.54	5.3 ± 0.33
2 min	1.6 ± 0.37	3.7 ± 0.67
3 min	0.63 ± 0.13	1.0 ± 0.43
Reoxygenation		
1 min	4.7 ± 0.48	9.3 ± 0.62[b]
2 min	6.0 ± 0.55	10.9 ± 0.90[b]
3 min	6.3 ± 0.25	12.1 ± 1.3[b]
5 min	5.0 ± 0.20	12.6 ± 1.2[b]

[a] Tyrode's solution did not contain glucose. Each value represents the mean ± SEM; $n = 4$ for steroid-treated group; $n = 4$ for vehicle-treated group.

[b] Indicates significant difference ($P < 0.05$) when methylprednisolone-treated group compared to vehicle-treated group.

tively, both approximately 5% of the prehypoxic values. During the reoxygenation period, hearts from methylprednisolone-treated rats develop significantly more tension ($P < 0.05$) than do hearts from vehicle-treated rats. The methylprednisolone-treated group regains a maximum force of contraction of 12.6 ± 1.2 g (84% of the prehypoxic value) whereas the vehicle-treated group attains a force of contraction equivalent to 6.3 ± 0.25 gm (44% of the prehypoxic value) at this same time period.

Discussion. The results of this study clearly demonstrate that pretreatment with a pharmacologic dose of the glucocorticoid, methylprednisolone, improves the functional recovery of the isolated hypoxic rat heart during a period of reoxygenation. The fact that the capacity of the heart to recover after hypoxia is better preserved in the methylprednisolone-treated group suggests that this steroid prevents some of the deleterious effects that are produced by hypoxia.

The precise mechanism by which methylprednisolone enhances cardiac recovery of the hypoxic rat heart is not clear, however, the protective mechanism may be related to the ability of methylprednisolone to reduce the amount of cardiac cellular damage as has been reported by other investigators (3, 4, 7). One of the primary causes of cellular damage during hypoxia is the disruption of lysosomal membranes with a release of acid hydrolases into the cytoplasm of the cell (8). Glucocorticoids have been shown to stabilize lysosomal membranes thus making them more resistant to an hypoxic stress (9). This lysosomal membrane stabilization would be expected to result in a decreased cardiac cellular autolysis and in an improved cardiac performance upon reoxygenation.

Although glucocorticoids have been reported to increase glucose transport and thus enhance the intracellular flux of this energy source (10), the present study does not support this process as the mechanism whereby methylprednisolone affords protection to the hypoxic heart. The data presented herein illustrate that methylprednisolone protects the isolated rat heart even when glucose was omitted from the perfusion medium. However, it was noted that hearts perfused with glucose containing Tyrode's solution did perform better during and after the hypoxic insult. Exogenous carbohydrates supplied to isolated hearts have been shown to help maintain available levels of high energy phosphates (11). Therefore, the improved contractile performance during hypoxia seen in these hearts perfused with 5 m*M* glucose appears to result from the availability of exogenous glucose. However, the improved contractile performance due to increased availability of glucose does not explain why the hearts of steroid-treated rats perfused with glucose-free medium performed better than vehicle-treated hearts. Regardless of the presence or absence of glucose, methylprednisolone produced this protective effect which suggests that the protective effect of this hormone may be unrelated to increased utilizable energy sources accessible to the heart.

Other protective effects of corticosteroids which have been proposed include: (a) decreases in peripheral vascular resistance (12), (b) prevention of the formation of a myocardial depressant factor (MDF) (13) and (c) increases in cardiac output, coronary blood flow and cardiac efficiency (14, 15). Since the experimental design of the present study has excluded such extracardiac factors as MDF and peripheral vascular resistance it appears that the cardiac protection seen in these ex-

periments was not due to a modulation of these factors. The above reports which have suggested that steroids exert a direct protective effect on the heart have been controversial. Lefer (16) was unable to elicit a positive inotropic effect in the intact cat heart after 20 mg/kg of methylprednisolone. The data from our study support this finding in that methylprednisolone did not exert a positive inotropic effect on the isolated rat heart under control conditions. This is further evidenced by the similar force of developed tension in hearts of both vehicle-treated and methylprednisolone-treated rats. Furthermore, although not shown, there were no differences in total coronary blood flow between the two groups of hearts suggesting that a change in total coronary blood flow was not the mechanism for the enhanced performance in the methylprednisolone pretreated group. However, no measurements were made testing the possibility of a redistribution of coronary blood flow which may have benefited the myocardium.

Although we have not elucidated the precise mechanism for the protective action of the glucocorticoid, methylprednisolone, on the hypoxic heart, it has been shown that this steroid does attenuate some of the deleterious effects of hypoxia and thus enhances the functional recovery of the hypoxic heart.

Summary. The isolated rat heart was studied to determine if pretreatment with the glucocorticoid, methylprednisolone (30 mg/kg), both afforded protection to the heart during a period of hypoxia and improved recovery during reoxygenation. Hearts from rats treated with methylprednisolone sodium succinate exhibited significantly better force of contraction during the period of reoxygenation than did hearts from vehicle-treated rats. This improved recovery of the steroid-treated group was seen in the presence or absence of glucose and was not related to a steroid-induced positive inotropism or enhanced total coronary flow. However, the possibility exists that methylprednisolone pretreatment was associated with a favorable redistribution of coronary blood flow and as such produced a protective effect on the hypoxic heart.

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