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THE POSITIVE INOTROPIC ACTION OF INSULIN IN THE CANINE HEART *

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The effects of glucagon-low insulin were studied on canine isolated papillary muscles incubated in Chenoweth Kolle solution and in the pentobarbital anesthetized dog. Insulin, 12.5 mU/ml, increased developed isometric tension of papillary muscles which had equilibrated for 17 hrs, but not in muscles incubated up to 60 min. The decrease in isometric tension produced by propranolol, 10^{-6} M, but not that due to pentobarbital sodium, 80 μg/ml, was reversed by insulin. The positive inotropic effect of insulin was obtained when pyruvate, fumarate and glutamate replaced glucose as substrate in the Chenoweth Kolle solution. The intracoronary administration of insulin, 0.1 U/kg, increased left ventricular isometric tension and the rate of tension development in dog hearts depressed with propranolol, 2 mg/kg. Insulin exerts a direct positive inotropic effect upon canine heart muscle depressed by propranolol or due to prolonged incubation. The effect is not dependent upon catecholamines, glucagon or glucose. The positive inotropic effect is not observed in non-depressed hearts or in barbiturate depressed hearts.

Insulin (glucagon-low) Heart failure Myocardial substrates
Propranolol Inotropic action

1. INTRODUCTION

Early studies by Farah (1938) revealed that certain preparations of insulin produced positive inotropic effects upon the canine heart; an observation which was attributed to contamination of the hormonal preparation by an unknown factor or factors. Similarly, Regan and coworkers (1963), who used a purified preparation of insulin (glucagon-free insulin, Eli Lilly), concluded that the pancreatic hormone did not possess inotropic effects when injected into the left coronary artery of the anesthetized dog, even though insulin exhibited its classical effect on glucose uptake and an increase in the cardiac respiratory quotient.

Renewed interest in the possible inotropic effect of insulin has been stimulated by the studies of Sodi-Pallares et al. (1963) who have advocated the clinical use of insulin in combination with glucose and KCl in the management of patients with acute myocardial infarction. A recent study by Carlstrom and Karlefors (1970) has indicated that cardiac performance was increased in the juvenile diabetic patient after adequate insulin therapy. Sharma et al. (1970) and Taylor et al. (1969) have reported a suppression of insulin secretion to be associated with heart failure or myocardial infarction; the therapeutic implications of which should deserve serious consideration since the supression of insulin secretion may possibly aggravate the heart failure. Recent studies by Merin (1970) in-

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dicate that insulin could reverse the myocardial depression produced by halothane; an action that was attributed to the ability of insulin to enhance myocardial glucose transport. A similar conclusion regarding the inotropic action of insulin was arrived at by Weissler et al. (1970) who reported that the hormone increased glucose transport and cardiac performance in the isolated perfused rat heart. Majid et al. (1971) have demonstrated a correlation between the low cardiac output state and the inability to secrete insulin in response to the i.v. injection of tolbutamide in patients after open-heart surgery and suggest that the administration of insulin may possibly produce clinical improvement.

In view of the divergent views concerning the inotropic action of insulin and its mechanism, a study was undertaken to investigate the effects of the hormone on dog isolated papillary muscles as well as in the intact heart of the dog. The results suggest that insulin is capable of significantly augmenting contractile force of the depressed myocardium and that the positive inotropic effect is not dependent upon increased glucose transport across the myocardial cell.

2. MATERIALS AND METHODS

2.1. *Papillary muscle preparation*

Mongrel dogs, 7–10 kg in weight were anesthetized with pentobarbital sodium, 30 mg/kg, i.v., and 90 min were allowed to elapse before thoracotomy was performed. This time interval was considered sufficient to permit adequate distribution of the barbiturate anesthetic and a reversal of the transient depression of myocardial contractility that occurs upon the rapid i.v. administration of pentobarbital sodium. The heart was rapidly excised and placed in cooled, oxygenated Chenoweth–Kolle solution. Right ventricular papillary muscles were carefully selected and mounted in muscle chambers containing 50 ml of Chenoweth–Kolle solution which was vigorously bubbled with 95% O_2 – 5% CO_2 and maintained at a temperature of 37°C. The nontendenous ends of the muscles were held in a Lucite clip and the upper end of the muscles were attached by braided non-capillary silk to a FT-03 force displacement transducer. The muscles were stretched by a resting force which was near the peak of their active length–tension curves (approximately 2 g) and were maintained at this length throughout the study. The isometric muscles were stimulated via punctate electrodes with square wave pulses of 0.5 msec duration, at a rate of 60 min^{-1} with a current 10% above threshold delivered from an 104A American Electronics Laboratories Stimulator. All recordings were made on a Grass Model 7 Polygraph. The signal from the force displacement transducer was simultaneously displayed on a Tektronix 502A oscilloscope and photographed with a Polaroid camera.

The muscles were divided into two groups. Those of group I were allowed to contract isometrically for 1 hr before being exposed to insulin. The papillary muscles in group II were allowed to contract isometrically for a period of 17 hr before the addition of insulin. Groups I and II muscles will be referred to as 'fresh' and 'fatigued' muscles, respectively. The canine papillary muscles were selected with care so that only the smallest muscles would be used so as to insure adequate oxygenation of the muscle. The papillary muscles varied from 2 to 3 mm diameter and were from 7 to 10 mm in length. The reason for using the canine preparation rather than the cat or rabbit was because the latter two species did not exhibit an inotropic response to insulin. The adequacy of the canine papillary muscle preparation is shown by the fact that the muscles continued to function for periods in excess of 20 hr and responded in a typical manner to paired electrical pacing and to ouabain.

The composition of the bath medium was as follows expressed as mM: NaCl, 120; KCl, 5.8; $CaCl_2 \cdot 2H_2O$, 2.2; $MgCl_2 \cdot 6H_2O$, 2.1; glucose, 10; and $NaHCO_3$, 14. The pH was adjusted to 7.4. In addition, two other bath media were prepared in which the dextrose was reduced to 1 mM and a solution without dextrose, but to which had been added: potassium pyruvate, 5 mM, sodium fumarate, 5 mM, and mono-sodium glutamate, 5 mM. The latter two solutions are referred to respectively as a 'dextrose-low' and a 'dextrose-free solution'. In all instances trypsin-treated bovine insulin, low in glucagon, was added to the muscle chamber in cumulative doses to attain concentrations of 12.5, 25.0, 50.0 and 100 mU/ml. Successive additions of insulin were made at 10 min intervals. At the conclusion of each experiment the papillary muscles were subjected to paired-pacing to determine the maximum isometric

tension for each muscle. The isometric force responses to insulin were expressed as a percent of the maximum isometric force. The data were analyzed statistically with the standard *t*-test for group comparisons (Snedecor and Cochran, 1967).

2.2. *Intact dog heart preparation*

Male mongrel dogs, between 13 and 27 kg, were anesthetized with pentobarbital sodium, 30 mg/kg, i.v. Positive pressure respiration was maintained through an endotracheal tube by a Harvard respirator pump. Systemic arterial pressure was measured from a femoral artery with a Statham pressure transducer. The cervical vagi were severed bilaterally. The heart was exposed through a thoracotomy in the left fifth intercostal space. A 5–10 mm segment of the anterior descending branch of the left coronary artery was dissected free near its origin. Heparin was administered in an initial dose of 5 mg/kg and then in hourly doses of 2.5 mg/kg. The anterior descending coronary artery was cannulated with a polyethylene cannula and perfused from a femoral artery. The blood flow through the descendans branch was maintained constant by a bilateral roller pump. In each experiment the descendans coronary flow was adjusted to produce a mean coronary perfusion pressure equal to the mean systemic arterial pressure. Coronary perfusion pressure was measured with a Statham pressure transducer from a sidearm of the coronary artery cannula near the heart. The temperature of the perfusing blood was maintained constant at 37°C by means of a heat exchanger interposed between the perfusion pump and the heart. The temperature was monitored continuously with a thermistor probe inserted into the blood stream near the heart. A calibrated Brodie–Walton strain gauge arch was sutured at the apex of the left ventricle in a region supplied by the anterior descending coronary artery. The peak rate of force development (dF/dt) was electronically differentiated and recorded simultaneously with isometric ventricular tension on a Grass Model 7 Polygraph.

All drugs were administered either intravenously into a cannulated external jugular vein, or by intracoronary (i.c.) injections via a side arm of the extracorporeal circulation supplying the anterior descending coronary artery.

3. RESULTS

3.1. *Effects of insulin upon dog isolated papillary muscles*

A total of 14 papillary muscles was incubated in Chenoweth–Kolle solution containing dextrose in a concentration of 180 mg% (10 mM). The muscles were allowed to contract isometrically for 1 hr before insulin was added to the bath medium. The control developed isometric tension averaged 0.58 ± 0.11 g which was 23.4 ± 2.9% of the maximum isometric tension developed in response to paired pacing. The addition of insulin, 12.5 mU/ml, produced an average developed tension of 0.70 ± 0.15 g or 29.5 ± 5.3% of the maximum developed tension. The insulin-induced change in developed tension, or the mean change in tension expressed as a percent of the maximum tension, did not differ significantly from the control values ($p>0.05$). When papillary muscles were allowed to contract isometrically for 17 hr in Chenoweth–Kolle solution (dextrose, 180 mg% or 10 mM) the developed tension in 10 preparations averaged 0.12 ± 0.02 g or 13.1 ± 3.1% of the maximum developed isometric tension. The addition of insulin, 12.5 mU/ml, to the 'fatigued' papillary muscles produced a significant increase in isometric tension as expressed in absolute units or as a percentage of maximum developed tension: 0.24 ± 0.5 g ($p<0.025$) and 21.3 ± 3.5% ($p<0.005$), respectively. Qualitatively similar results were obtained when the dextrose concentration was reduced to 18 mg% (1 mM) or when the dextrose was eliminated and replaced with pyruvate, fumarate and glutamate. The inotropic response to insulin was observed only in 'fatigued' papillary muscles. The data are summarized in table 1 and the insulin-induced responses upon 'fatigued' papillary muscles incubated in the presence of 1 mM dextrose (18 mg%) or in the absence of dextrose are shown in figs. 1 and 2, respectively.

The insert in fig. 1 shows the isometric force responses as recorded from an oscilloscope before and after the addition of insulin to the bath medium. Although insulin increased the developed isometric tension and rate of tension development, it did not alter the time to peak tension or change the duration of the contractile response.

It should be noted that although the papillary muscles were depressed after 17 hr of incubation, in-

Table 1

Effect of insulin upon canine papillary muscles.

Buffer solution (n)	Treatment	Isometric tension (g)	p value	Change in tension (% of maximum)	p value
		Values obtained 60 min after incubation; mean ± S.E.M.			
Glucose, 180 mg% (14)	None	0.58 ± 0.11	> 0.05	23.4 ± 2.9	> 0.05
	Insulin, 12.5 U/ml	0.70 ± 0.15		29.5 ± 5.3	
Glucose, 18 mg% (10)	None	1.03 ± 0.26	> 0.05	24.4 ± 2.8	> 0.05
	Insulin, 12.5 mU/ml	0.97 ± 0.12		23.1 ± 2.8	
Glucose, 0 mg% Pyr Fum Glu* (9)	None	0.66 ± 0.15	> 0.05	31.3 ± 4.6	> 0.05
	Insulin, 12.5 mU/ml	0.65 ± 0.12		32.8 ± 4.7	
		Values obtained 17 hr after incubation; mean ± S.E.M.			
Glucose, 180 mg% (10)	None	0.12 ± 0.02	< 0.025	13.1 ± 3.1	< 0.005
	Insulin, 12.5 mU/ml	0.24 ± 0.05		21.3 ± 3.5	
Glucose, 18 mg% (7)	None	0.50 ± 0.09	< 0.01	10.4 ± 1.9	< 0.005
	Insulin, 12.5 mU/ml	1.04 ± 0.18		21.0 ± 2.3	
Glucose, 0 mg% Pyr Fum Glu* (7)	None	0.31 ± 0.07	< 0.025	19.3 ± 5.0	< 0.01
	Insulin, 12.5 mU/ml	0.71 ± 0.20		38.2 ± 7.4	

* pyruvate - fumarate - glutamate.

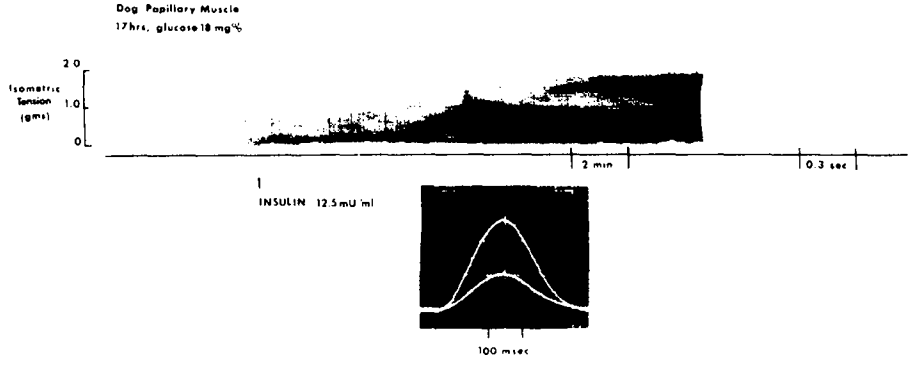

Fig. 1. Effect of insulin, 12.5 mU/ml, upon developed isometric tension in the dog papillary muscle incubated for 17 hr in Chenoweth–Kolle solution having a glucose concentration of 18 mg% (1 mM). The inset at the bottom of the figure is the photographic recording of the isometric contractions recorded from an oscilloscope. The onset of the positive inotropic effect requires approximately 2 min and reaches a maximum within 10–12 min. Insulin increased the rate of tension development, without changing the time to peak tension or the duration of the contractile event.

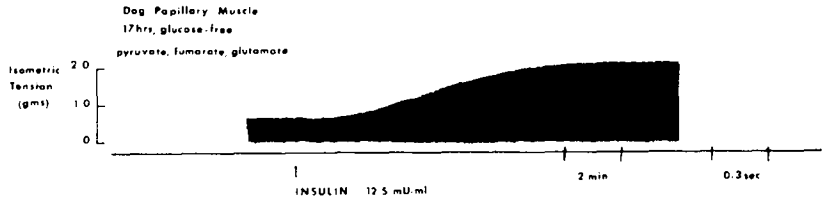

Fig. 2. Effect of insulin, 12.5 mU/ml, upon developed isometric tension in the dog papillary muscle incubated for 17 hr in glucose-free Chenoweth–Kolle solution in which pyruvate, fumarate and glutamate served as substrates. The contractile response to insulin appears within 2 min and reaches a maximum within 10–12 min.

sulin was capable of producing an average isometric force response equal to that produced by papillary muscles incubated for 1 hr in 18 mg% glucose or glucose-free Chenoweth–Kolle solution. Although insulin produced a positive inotropic effect in 'fatigued' papillary muscles incubated in the presence of 180 mg% glucose, the magnitude of the response was not as great as that obtained with muscles incubated in the presence of 18 mg% glucose or in the presence of pyruvate, fumarate and glutamate. Therefore, the positive inotropic response to insulin was observed only in papillary muscles permitted to contract continuously for 17 hr and the insulin-induced response was independent of the presence of glucose in the medium. It was also noted that the response to insulin occurred at a concentration of 12.5 mU/ml. Lower concentrations failed to produce a significant augmentation of contractile force and further increases in the concentration did not produce an additional increment in isometric tension. The increment in developed tension was sustained and was slowly reversed by washing the muscle for several hours during which time the addition of insulin to the bath failed to initiate a significant positive inotropic response.

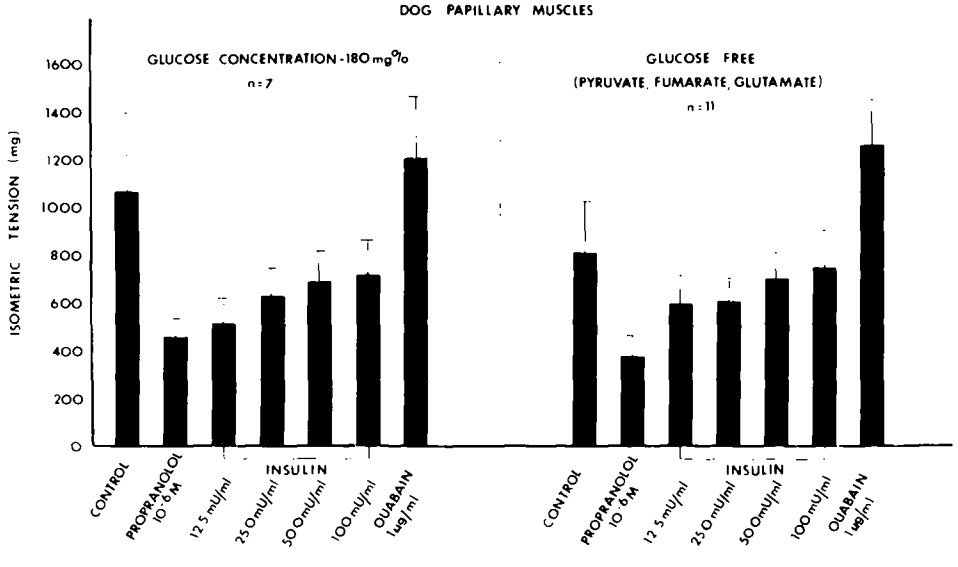

Fig. 3. The effect of insulin on the propranolol-depressed dog papillary muscle. Muscles were exposed to propranolol, 10^{-6} M, for 45 min before the addition of insulin. Under these conditions, the muscles displayed a dose-dependent response to insulin and a further augmentation of developed tension in response to ouabain.

3.2. *Effect of insulin upon dog isolated papillary muscles depressed with propranolol or pentobarbital sodium*

Since the previous studies had suggested that insulin would exert a positive inotropic effect upon 'fatigued' papillary muscles, it was considered important to study the effect of the hormone in the presence of drugs known to produce a depression of myocardial contractility.

Recently excised papillary muscles, incubated in the presence of glucose (180 mg%) or in a glucose-free medium were exposed to d,l-propranolol, 10^{-6} M, for a period of 45 min. The addition of d,l-propranolol resulted in a significant depression of isometric tension. The addition of insulin, 12.5 to 100 mU/ml, produced a dose-dependent increase in isometric force in both groups of muscles. An additional increment in isometric tension was achieved by the administration of ouabain, 1 μg/ml. The data are presented graphically in fig. 3. Unlike the study in non-depressed papillary muscles, insulin produced an augmentation of contractility in muscles which had been contracting isometrically for less than 2 hr, but which had been depressed by d,l-propranolol added to the bath medium. Another point of difference, was the fact that papillary muscles depressed with dl-propranolol exhibited a dose-dependent increase in isometric tension in contra-distinction to 'fatigued' muscles which failed to show a dose-dependent change in developed tension.

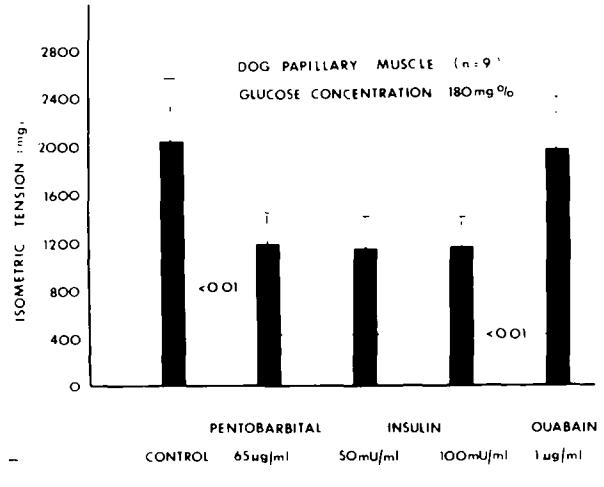

Fig. 4. The effect of insulin on the pentobarbital-depressed dog papillary muscle. The addition of pentobarbital produced a significant depression in isometric force which could not be reversed by insulin. Ouabain, however, produced a positive inotropic effect.

The results obtained with insulin in 9 papillary muscles depressed with pentobarbital sodium are shown in fig. 4. Unlike the previous results, insulin failed to augment the contractility of the pentobarbital-depressed muscle even though the preparations did exhibit a significant increase in developed isometric force when exposed to ouabain, 1 μg/ml.

Insulin heated to 100°C at pH 11 for 30 min failed to augment developed isometric force although the subsequent administration of the non-denatured hormone to the isolated muscle produced the typical positive inotropic effect.

3.3. *Effect of insulin upon the intact canine heart*

Insulin in a dose of 0.1 U/kg was administered into the anterior descending coronary artery in each of 6 open-chest anesthetized dogs, which had been treated with propranolol, 2 mg/kg, i.v. In each of the dogs, isometric myocardial force and the rate of tension development (dF/dt) recorded from the descendans region were significantly reduced after the administration of propranolol. The subsequent administration of insulin (0.1 U/kg) directly into the anterior descending coronary artery was associated with a positive inotropic effect characterized by an increase in isometric force and dF/dt. The results from one experiment are shown in fig. 5 and the data are summarized in fig. 6. The mean control ± S.E.M. isometric force and dF/dt values were 61.3 ± 6.7 g and 1801.8 ± 264.3 g/sec, respectively. After propranolol both values were significantly reduced to 45.0 ± 3.1 g and 1128.3 ± 160.8 g/sec. Insulin restored both parameters to the control state 61.3 ± 5.9 g and 1464.9 ± 227.2 g/sec, respectively. The peak response to insulin occurred within 5 min and isometric force and dF/dt had returned to the pre-insulin level by the end of 20 min. As observed with the isolated muscle preparations, the response to insulin was delayed and did not begin until 1 or 2 min after administration of the hormone.

In 3 of the experiments summarized in fig. 6, a second strain gauge arch was sutured to the right ventricular wall for the measurement of right ventricular isometric force. The positive inotropic response to insulin was noted to occur only in the region of the

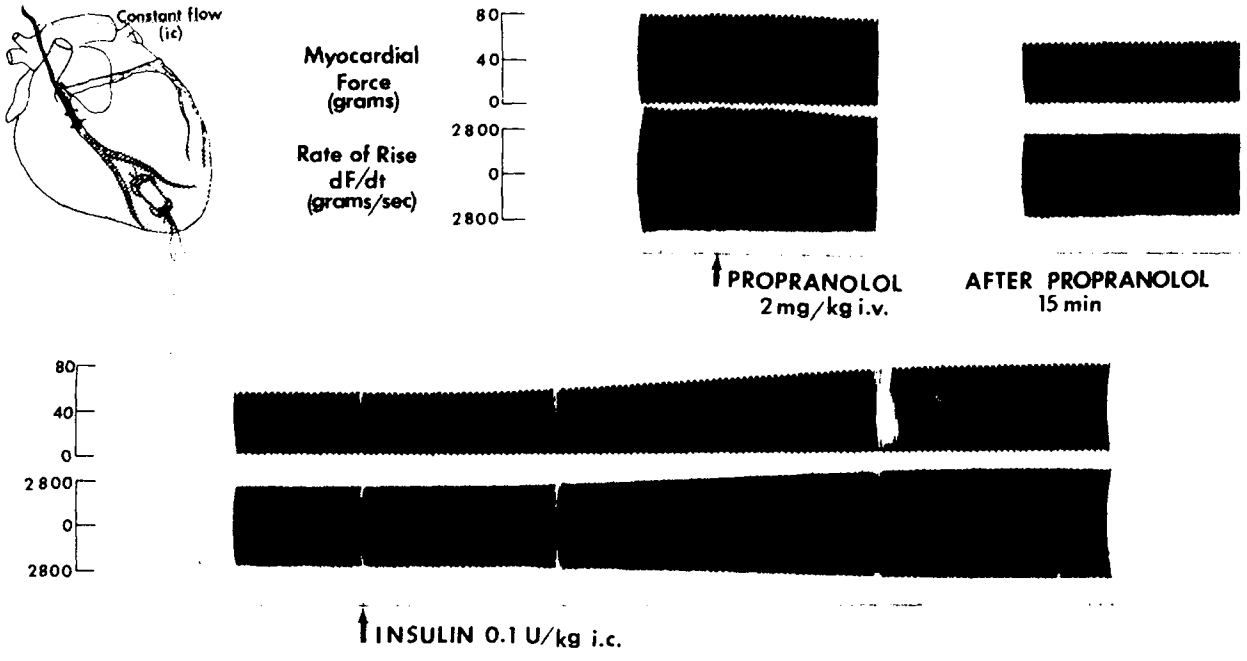

Fig. 5. The reversal of myocardial depression in the intact dog heart by the intracoronary administration of insulin 0.1 U/kg. Both myocardial isometric tension and dF/dt increased within 1–2 min after the administration of the hormone. The anterior descending coronary artery was supplied with arterial blood by means of a roller pump and left ventricular isometric force was recorded from the region of the heart supplied by the anterior descending coronary artery.

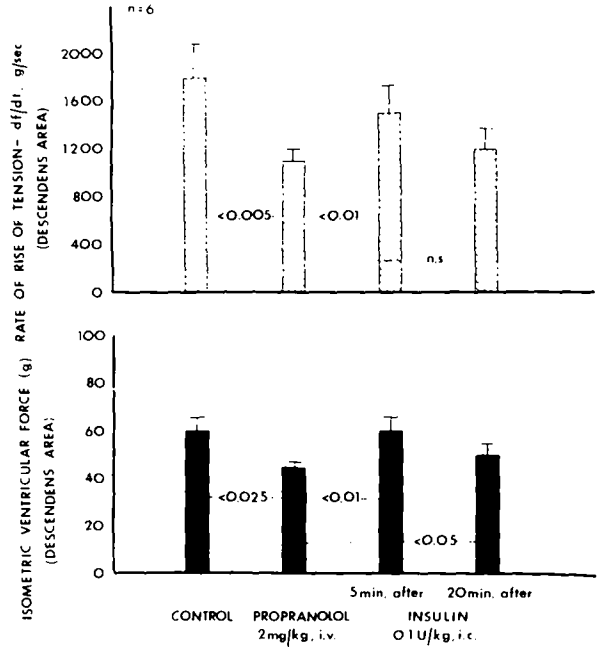

Fig. 6. Summary of the results obtained in 6 dogs illustrating the positive inotropic action and time course of insulin in the pentobarbital depressed heart. The inotropic response had subsided by the end of 20 min.

heart perfused by the anterior descending coronary artery; the ismetric force recorded from the right ventricle did not change after insulin although it was significantly decreased after intravenous administration of propranolol. These results suggest that the positive inotropic action of insulin is the result of a direct effect upon the myocardium and is not dependent upon the release of neurotransmitters or other biochemical changes brought about by the hormone. Furthermore, the presence of propranolol, 2 mg/kg, rules out the possibility that the response is due to myocardial β-adrenergic stimulation. Insulin did not produce any alteration in heart rate which would suggest that glucagon is not involved in the insulin-induced response, since glucagon is known to produce both positive inotropic and chronotropic effects.

4. DISCUSSION

Recent investigations have provided evidence to suggest that insulin, by virtue of its ability to facilitate glucose transport across the myocardial cell, can

improve cardiac performance (Sheldon et al., 1969; Merin, 1970; Weissler et al., 1970; Henderson et al., 1970). The positive inotropic action of insulin has been demonstrated in the present study in the dog isolated papillary muscle which was allowed to beat continuously for a period of 17 hr at which time the muscles exhibited a marked depression of contractile force. Insulin was capable of restoring the contractile force of depressed muscles to levels of tension development which approximated those of non-depressed muscles. Papillary muscles incubated for 17 hr in the presence of 180 mg% glucose showed a greater degree of depression than did papillary muscles incubated for the same period of time in the presence of 18 mg% glucose or in the absence of glucose. In the latter instance, tricarboxylic acid metabolites served as substrates. The augmentation of isometric tension upon the administration of insulin was more pronounced in the latter two groups, whereas, the positive inotropic response in muscles incubated in the presence of 180 mg% glucose, while statistically significant, never restored tension development to a level comparable to that of non-depressed papillary muscles. The results demonstrate that glucose alone as substrate, in a concentration of 180 mg%, is not capable of maintaining the contractile state of the papillary muscles and that contractile performance under these conditions is not restored to control levels by insulin. In constrast was the observation that insulin was more effective in restoring contractile tension in the absence of extracellular glucose. These results suggest that the inotropic action of insulin in the depressed papillary muscle is not related to its ability to facilitate the transport of glucose across the myocardial cell and that the pancreatic hormone may affect other metabolic processes which alter myocardial contractility. Furthermore, the inotropic action of insulin in the dog papillary muscle is not demonstrable in recently excised, non-depressed papillary muscle preparations. It should be noted however, that under anaerobic conditions the positive inotropic action of insulin is observed only when glucose is present in the bathing medium (MacLeod and Prasad, 1969; Prasad and Callaghan, 1969) a result which is not unexpected since the glycolytic pathway would be the primary source of energy production under conditions of oxygen deprivation. However, the inotropic response to insulin as reported by MacLeod and Prasad (1969) and Prasad and Callaghan (1969) was a minimal effect and did not approach the contractile level which existed in the control state.

It has been suggested that the positive inotropic response to glucose which is further enhanced by insulin occurs in rat heart under conditions in which oxygen is limited (Henderson et al., 1970). This is supported by the previously mentioned observations by MacLeod and Prasad (1969) and by Prasad and Callaghan (1969). It is doubtful that oxygen limitation was a factor in the present experiments since the contractile performance of the 'fatigued' papillary muscles was restored to normal upon the addition of insulin in those muscles incubated in 18 mg% glucose and in the group of muscles incubated in a glucose-free medium in which pyruvate, fumarate and glutamate served as substrates. It is unlikely that oxygen-limited muscles would have been able to achieve this level of contractile activity let alone survive a continuous period of stimulation in excess of 17 hr. As integrity of cell structure is believed to be essential for an effect of insulin, it is not likely that it would have exerted an effect under prolonged hypoxic conditions. It is unlikely that endogenous lipid serves as an energy source for cardiac muscle except during intense exercise (Rinetti et al., 1954) or prolonged fasting (Denton and Randle, 1967). Therefore, under conditions of high concentrations of extracellular glucose, the need for endogenous lipid as substrate is diminished. The limited effect of insulin under these conditions might be attributed to the fact that its primary metabolic action in bringing about a positive inotropic effect does not involve glucose uptake and the glycolytic pathway. The inotropic response is best observed under conditions in which lipid or carboxylic acid metabolism serves as the primary energy source. This situation would prevail when extracellular glucose is diminished or absent. It has been reported that glucose uptake by heart muscle is non-maximal at an extracellular concentration of 5 mM; but is maximal at 20 mM (Penney and Cascarano, 1970). The results of the present study do not rule-out the important role of insulin upon glucose transport, but suggest that a second mechanism involving the oxidative pathway might play a significant part in the hormone's inotropic action.

The failure to observe an inotropic response to insulin in the barbiturate depressed heart muscle

preparation is difficult to explain at present. Similarly, why insulin should reverse the negative inotropic action of propranolol is not clearly understood. Although the effects of propranolol upon myocardial metabolism have not been clearly elucidated, there is some evidence to suggest that propranolol interferes with myocardial lipid metabolism (Satchell et al., 1968; Masters and Glaviano, 1969). Furthermore, Bewsher et al. (1966, 1967) have provided evidence to suggest that an antogonism exists between insulin and β-adrenergic receptor blocking agents such as pronethalol and propranolol when studied upon adipose tissue. A similar mechanism may be involved in myocardial tissue and may account for the dose-dependent increase in isometric force observed in the propranolol depressed papillary muscle.

The present investigation has demonstrated that glucagon-low insulin is capable of augmenting myocardial contractility in the depressed heart and that the response is not dependent upon the ability of the hormone to facilitate glucose transport across the myocardial cell. The potential therapeutic importance of these observations is suggested by the recent publications in which the role of insulin in heart failure and in patients with myocardial infarction has been discussed (Carlstrom and Karlefors, 1970; Taylor et al., 1969; Sharma et al., 1970; Taylor, 1971; Majid et al., 1971). Thus, insulin may play an important role in maintaining mechanisms of energy production in the failing myocardium and further studies on the metabolic effects of the hormone in experimental congestive heart failure should be undertaken.

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