Use of Mannitol to Obviate Propranolol-Induced Myocardial Depression in Ischemic Canine Heart

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Abstract □ To obviate the myocardial depressant action of propranolol, the use of this drug in combination with a positive inotropic agent, mannitol, was studied in the ischemic heart of the dog. Mannitol (25%) administered prior to propranolol augmented myocardial contractility, as shown by an upward shift of the relationship between the pressure change with time and the mean left atrial pressure. Subsequent administration of propranolol did not offset the positive inotropic response to mannitol whereas it markedly reduced the heart rate from a control ischemic value of 127.8 ± 3.4 to 109 ± 4.0 beats/min. The myocardial oxygen requirement, as determined by the tension–time index per minute, decreased 26.3 ± 1.2% below the control ischemic value. These findings suggest that hypertonic mannitol augments contractility in the ischemic failing heart while sustaining the desirable negative chronotropic action of propranolol. This procedure may potentiate the beneficial effects of propranolol on the myocardial oxygen balance and serve as an effective therapeutic modality in the control of acute irreversible ischemia.

Keyphrases □ Propranolol—β-adrenergic blocking activity, effect on myocardial contractility, oxygen requirement, and heart rate, combined action of propranolol and hypertonic mannitol, hemodynamic changes in dogs □ β-Adrenergic blocking activity—effects of propranolol on ischemic failing heart, effects of hypertonic mannitol, dogs □ Myocardial depressants—propranolol, effects of hypertonic mannitol, β-adrenergic blocking activity, dogs

Propranolol, a β-adrenergic blocking agent, was introduced for clinical use in 1968. The value of this drug in the control of reversible ischemia (angina) has been established and reviewed extensively (1). Reversible myocardial ischemia suggests that there is a temporary imbalance between the myocardial oxygen supply and demand and the inability of the coronary vessels to provide adequate oxygenated blood. If the oxygen supply cannot be improved, the only way to establish the myocardial oxygen balance is to reduce the oxygen demands of the heart. Propranolol achieves this goal by reducing the heart rate and cardiac contractility.

The role of β-adrenergic blockade in the treatment of irreversible myocardial ischemia (acute myocardial infarction) has been controversial. β-Adrenergic blockade has been demonstrated to reduce the area of myocardial ischemia in the dog heart following experimental occlusion of the anterior descending coronary artery (2, 3). Studies in humans also have provided evidence that propranolol may reduce the area of ischemia, as evidenced by a reversal of the S-T segment elevation during acute myocardial infarction (4). Similar results were obtained using the cardioselective β-blocking drug practolol (5).

Nevertheless, most reports have implied that β-adrenergic blockade is contraindicated in acute myocardial infarction because it decreases myocardial contractility and may aggravate or precipitate heart failure (6–8). Accordingly, the present study was undertaken to determine whether administration of a nonadrenergic positive inotropic agent, such as hypertonic mannitol (9–11), in conjunction with propranolol may prevent myocardial depression. A second objective was to evaluate the combined effects of mannitol and propranolol in myocardial oxygen requirements during generalized left ventricular ischemia in the intact canine heart. To date, the combined administration of hypertonic mannitol to obviate propranolol-induced myocardial depression in the intact dog has not been investigated. The results of these experiments could have important therapeutic implications.

EXPERIMENTAL

Healthy male and female mongrel dogs, 16–21 kg, were anesthetized by intravenous administration of chloralose (60 mg/kg). The trachea was intubated, and ventilation was instituted with a respiratory pump. The heart was exposed through a left thoracotomy at the fifth intercostal space by pericardiotomy, and the left main coronary artery was dissected free from the adjacent connective tissue.

Left ventricular pressure was recorded with a transducer attached to a polyethylene catheter, which was introduced into the left ventricle through the apex. Mean systemic arterial and left atrial pressures were measured through polyethylene cannulas inserted *via* the carotid artery and left atrial appendage, respectively, and connected to transducers. The maximal rate of left ventricular pressure development was obtained with an R–C differentiator, the amplitude of which was a linear function of 70 Hz. All variables were recorded on a polygraph.

After all parameters had stabilized following the surgical procedures, the mean left atrial and mean systemic pressures, changes in the left ventricular pressure, and heart rates were obtained. Control coronary occlusions were performed to determine the effects of ischemia alone. Generalized left ventricular ischemia was produced by gradual closure of a coarse cotton snare surrounding the left main coronary artery. The magnitude of ischemia was monitored by assessing the left ventricular myocardial contractility, which was evaluated by relating the change in the left ventricular pressure to the mean left atrial pressure. The latter parameter is an approximation of the left ventricular end diastolic pressure (12), and it was selected to avoid the artifacts generated by undamped catheters. Alterations in contractility produced by various interventions were used to assess their effects on the severity of myocardial ischemia. It was assumed that changes in the myocardial oxygen requirements mediated by the infused drugs affected the magnitude of ischemia and, thereby, myocardial contractility.

Alterations in myocardial oxygen requirements were evaluated using the tension–time index, which was obtained by multiplying the mean aortic systolic pressure by the duration of the systole (13). Mean systolic arterial pressure was estimated by:

$$\text{mean systolic pressure} = a - (a - b/3) \qquad \text{(Eq. 1)}$$

where a represents the peak systolic pressure and b is the aortic valve closure pressure (14).

Twelve dogs were divided into two equal study groups. In the first group, the effects of propranolol (0.5 mg/kg iv) in the ischemic failing heart were studied. After the onset of ischemia in the second group, the dogs were treated with hypertonic mannitol (25 g/100 ml) infused through the cannulated femoral vein at 3.82 ml/min by an infusion pump, and the responses to mannitol alone were recorded. Following recovery, the effects of mannitol plus propranolol (0.5 mg/kg) were assessed.

Serum osmolality was checked before, during, and after mannitol infusion and was consistent with the expected level (17 mOsm above the control value). Each experimental animal was used as its own control before any intervention was made in each experiment. The control state was considered to be the ischemic stage before treatment. The magnitude of ischemia was monitored by the decline in myocardial contractility. The standard t test, as described by Snedecor and Cochran (15), was used for group comparisons; the t test for paired comparison was used as presented

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Table I—Effect of Partial Occlusion of the Main Left Coronary Artery on Left Ventricular Hemodynamics [a]

Parameter	Control	Ischemia	Change from Control, %
Heart rate, beats/min	106.5 ± 2.5	125.3 ± 3.0	16.8 ± 1.1
Mean systemic arterial pressure, mm Hg	105.6 ± 2.0	98.4 ± 2.3	6.8 ± 1.1
Mean systolic arterial pressure, mm Hg	117.8 ± 2.1	108.8 ± 2.0	−7.6 ± 0.7
Mean left atrial pressure, mm Hg	4.8 ± 0.7	10.7 ± 1.5	122.8 ± 3.3
Ventricular ejection time, msec	197.1 ± 3.4	221.7 ± 3.2	12.6 ± 1.5
Maximal rate of left ventricular pressure development (*dp/dt*), mm Hg/sec	2633 ± 108.3	2298 ± 109.4	14.5 ± 1.9

[a] All values are expressed as the mean ± *SEM* of 12 experiments, and $p < 0.001$ for each parameter.

Table II—Effect of Generalized Left Ventricular Ischemia on Tension-Time Index [a]

Parameter	Control	Ischemia	Change from Control, %	*p*
Tension-time index per beat, mm Hg sec	23.2 ± 0.6	24.2 ± 0.6	4.5 ± 1.1	<0.01
Tension-time index per min, mm Hg sec/min	2502 ± 114	3057 ± 117	22.7 ± 1.9	<0.001

[a] Each value is expressed as the mean ± *SEM* of 12 experiments.

by Hill (16). All results are expressed with their standard error of the mean.

RESULTS

Ischemia—After partial occlusion of the left main coronary artery, myocardial contractility showed a rapid and pronounced decline (Fig. 1). As shown in Fig. 2, concurrent with the rise in the mean left atrial pressure (122.8 ± 3.3%), the left ventricular pressure fell (14.5 ± 1.9%); the heart rate increased from 106.5 ± 2.5 to 125 ± 3 beats/min whereas the mean systemic arterial pressure exhibited a moderate fall (6.8 ± 1.1%). The data are summarized in Table I. Table II shows the effect of generalized left ventricular ischemia on the tension-time index.

Propranolol—The hemodynamic changes following propranolol administration during generalized left ventricular ischemia are illustrated in Fig. 3. The mean left atrial pressure rose from an average control level of 8.9 ± 1.6 to 14.7 ± 2.3 mm Hg whereas the change in the left ventricular pressure decreased by 21 ± 2.5%, indicating a decline in cardiac contractility (Table III). Figure 4 depicts the myocardial depressant effect of propranolol in the ischemic failing heart. Although the heart rate fell substantially (17.9 ± 1.4%), the myocardial oxygen requirements did not decrease significantly, as evidenced by the tension-time index per minute. Propranolol evidently raised the tension-time index per beat by lengthening the systolic ejection time, as a result of which the tension-time index per minute failed to decrease (Fig. 5).

Mannitol—As shown in Fig. 4, infusion of hypertonic mannitol enhanced myocardial contractility in the ischemic heart; the mean left atrial pressure fell from the control ischemia value of 15.3 ± 2.1 to 6.7 ± 1 mm Hg whereas the change in the left ventricular pressure increased by 18.1 ± 1% (Table III). No significant changes in the heart rate and the mean systemic arterial pressure were noted. Although the heart rate remained essentially unaltered, the tension-time index per minute fell substantially (Fig. 5). By decreasing the systolic ejection time (12 ± 0.4%) (Table III), mannitol lowered the tension-time index per beat, thereby reducing the tension-time index per minute (Table IV).

Mannitol plus Propranolol—Administration of propranolol subsequent to mannitol infusion produced a progressive rise in the change in the left ventricular pressure (18.7 ± 0.7% above the control ischemia level) accompanied by a fall in the mean left atrial pressure (41 ± 4.5% below the control ischemia value), indicating a rise in myocardial contractility (Fig. 4). The heart rate fell from an average control ischemia value of 127.8 ± 3.4 to 109 ± 4 beats/min whereas the mean systemic arterial pressure remained essentially unaltered (Table III). The results clearly show that hypertonic mannitol may prevent the myocardial depressant action of propranolol (Fig. 4) but not its negative chronotropic effect (Fig. 3).

Direct comparison of the effects of mannitol and of mannitol plus propranolol in myocardial oxygen requirements were made in the same animals (Fig. 5). Propranolol administration subsequent to mannitol infusion greatly decreased the tension-time index per minute (26.7 ± 1.2% below the control ischemic value); this decrease was much greater than that achieved when mannitol was administered alone (9.7 ± 1.2%).

DISCUSSION

The present investigation demonstrates the ability of hypertonic mannitol to prevent the myocardial depressant action of propranolol in the ischemic failing canine heart without increasing the myocardial oxygen requirements. These results are in accord with reported observations (9) that infusion of hypertonic mannitol markedly improved ventricular function in the failing heart–lung preparation following occlusion of the left anterior descending coronary artery. The fact that the increased myocardial contractility was not accompanied by ECG S-T segment elevation suggested that the positive inotropic effects of mannitol are not oxygen wasting (9).

The effect of hypertonic mannitol on myocardial contractility is indicated by the rate of rise of the left ventricular pressure, an index of vigor of the left ventricular contraction. The change in the left ventricular pressure is determined mainly by the ventricular end diastolic volume and myocardial contractility (17). The decrease in the left ventricular pressure changes and the rise of the mean left atrial pressure manifested after the induction of ischemia indicate a depression of cardiac contractility (Fig. 1). As shown in Fig. 3, the rise in the left ventricular pressure changes that occurred concurrently with a fall in the mean left atrial pressure after mannitol infusion clearly shows the cardiotonic effects of mannitol in the ischemic failing heart.

The possible mechanisms by which hypertonic mannitol exerts its positive inotropic effects in the ischemic tissue are not known at present. It has been postulated that its osmotic action may decrease cellular edema

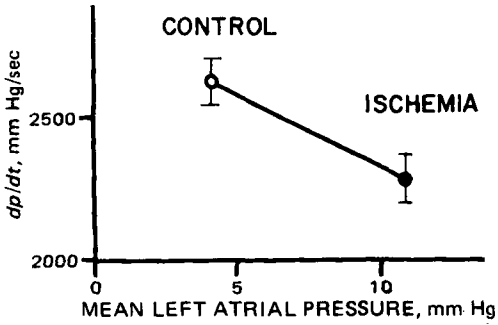

Figure 1—*Effect of partial occlusion of the main left coronary artery on myocardial contractility. The* x *axis shows the mean left atrial pressure, and the* y *axis shows the maximal rate of left ventricular pressure development* (dp/dt).

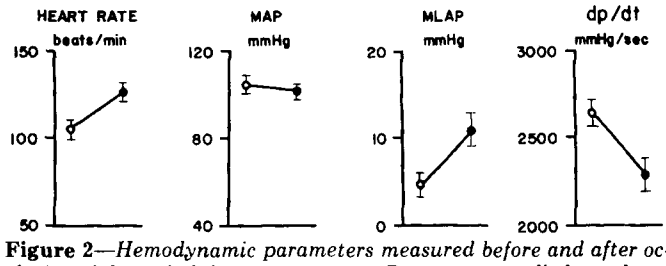

Figure 2—*Hemodynamic parameters measured before and after occlusion of the main left coronary artery. Parameters studied were heart rate, mean systemic arterial pressure (MAP), mean left atrial pressure (MLAP), and maximal rate of left ventricular pressure development* (dp/dt). *Key:* ○, *control; and* ●, *ischemia.*

Table III—Hemodynamic Changes [a] after Administration of Propranolol, Mannitol, and Propranolol plus Mannitol during Generalized Left Ventricular Ischemia

Condition	Heart Rate, beats/min	Mean Arterial Pressure, mm Hg	Mean Systolic Pressure, mm Hg	Mean Left Atrial Pressure, mm Hg	Ventricular Ejection Time, msec	*dp/dt*, mm Hg/sec
Control ischemia	122.7 ± 4.5	95.8 ± 3.0	106.8 ± 3.0	8.9 ± 1.6	220.8 ± 2.4	2086 ± 98.0
Ischemia plus propranolol	100.5 ± 3.1	92.3 ± 2.8	102.8 ± 2.7	14.7 ● 2.3	264.2 ± 3.0	1639 ± 60.2
Percent change from control	17.9 ± 1.4	3.6 ± 0.5	3.5 ± 0.6	74.3 ± 7.2	19.2 ± 1.8	21.0 ± 2.5
p	<0.005	<0.5	<0.2	<0.025	<0.001	<0.005
Control ischemia	128.0 ± 3.6	101.0 ± 2.9	107.2 ± 3.2	15.3 ± 2.1	222.0 ± 5.9	2510 ± 144.0
Ischemia plus mannitol	131.3 ± 4.5	99.8 ± 2.7	108.3 ± 3.0	6.7 ± 1.0	196.7 ± 5.9	2965 ± 177.0
Percent change from control	2.5 ± 1.3	1.1 ± 0.4	0.6 ± 0.3	46.0 ± 2.0	12.0 ± 0.4	18.1 ± 1.0
p	<0.5	<0.5	<0.5	<0.01	<0.001	<0.025
Postcontrol ischemia	127.7 ± 2.9	103.5 ± 3.3	111.0 ± 3.0	12.3 ± 1.7	227.0 ± 5.0	2554 ± 166
Control ischemia	127.8 ± 3.4	99.2 ± 2.5	109.0 ± 2.6	11.9 ± 1.9	220.0 ± 6.2	2534 ± 142
Ischemia plus propranolol plus mannitol	109.0 ± 4.0	97.0 ± 2.2	106.7 ± 2.2	6.7 ± 1.0	193.3 ± 4.9	3009 ± 170
Percent change from control	14.8 ± 1.4	2.1 ± 0.8	2.4 ± 0.6	41.0 ± 4.5	12.1 ± 0.5	187 ± 0.7
p	<0.005	<0.2	<0.2	<0.01	<0.005	<0.025
Postcontrol ischemia	129.3 ± 4.1	101.0 ± 2.2	109.0 ± 2.3	11.1 ± 1.9	222.0 ± 5.1	2515 ± 142

[a] Each value is expressed as the mean ± *SEM* of six experiments. [b] *dp/dt* = maximal rate of left ventricular pressure development.

and thereby enhance blood flow, which, in turn, may improve organ function (18). Ames *et al.* (19) stressed that edematous cells may impede blood flow within an organ and thus may impair nutritional circulation in the tissues. Summers and Jamison (20) used the silicone rubber technique to visualize the patency of the renal vasculature during ischemia to demonstrate, in the rat kidney, that cellular edema during transient ischemia may obstruct small vessels and thus intensify tissue damage. Apparently, the lumen of minute vessels, although narrowed by compression from the surrounding edematous cells, permitted the passage of the silicone rubber particles but not of the erythrocytes. Intravascular injection of hypertonic mannitol reestablished a normal vascular pattern, reduced tissue injury, and improved renal function (20). Osmotic shrinkage of the swollen cells presumably reduced the extravascular pressure exerted on the vascular bed, thus enhancing the blood flow.

Although hypertonic mannitol augmented collateral as well as total coronary blood flow in experimentally induced ischemia (9), the increase observed in myocardial contractility may not be totally dependent on changes in myocardial perfusion. It was demonstrated recently that hypertonic mannitol also exerts a positive inotropic effect in the isolated cat papillary muscle (11). The developed tension and the rate of tension rise were significantly greater when the papillary muscles were treated with mannitol regardless of whether mannitol was introduced before or later in the hypoxic period (11).

It has been postulated that a decrease in the intracellular volume produced by the osmotic action of hypertonic mannitol may increase the degree of activation of the myocardial contractile elements. This increased contractile activity may be a result of an increased calcium con-

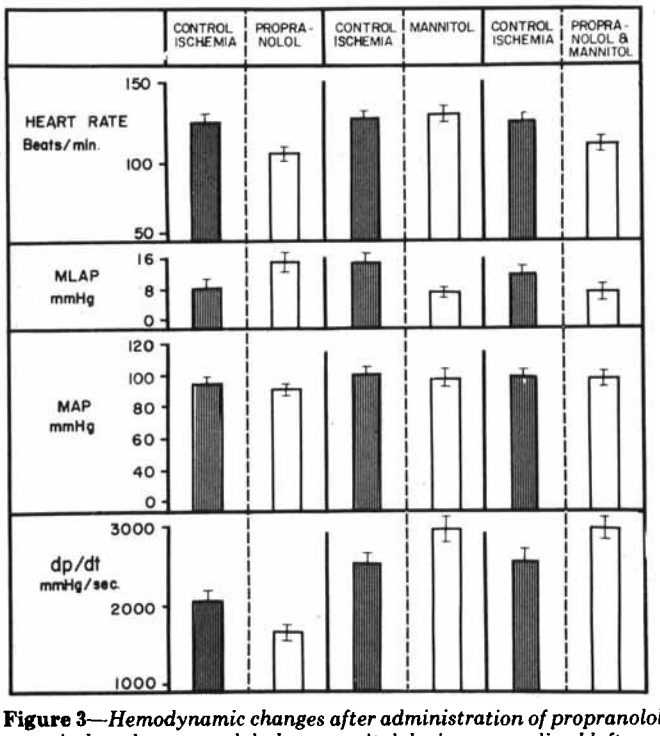

Figure 3—*Hemodynamic changes after administration of propranolol, mannitol, and propranolol plus mannitol during generalized left ventricular ischemia. Parameters measured are indicated on the y axis: heart rate, mean systemic arterial pressure (MAP), mean left atrial pressure (MLAP), and maximal rate of left ventricular pressure development* (dp/dt).

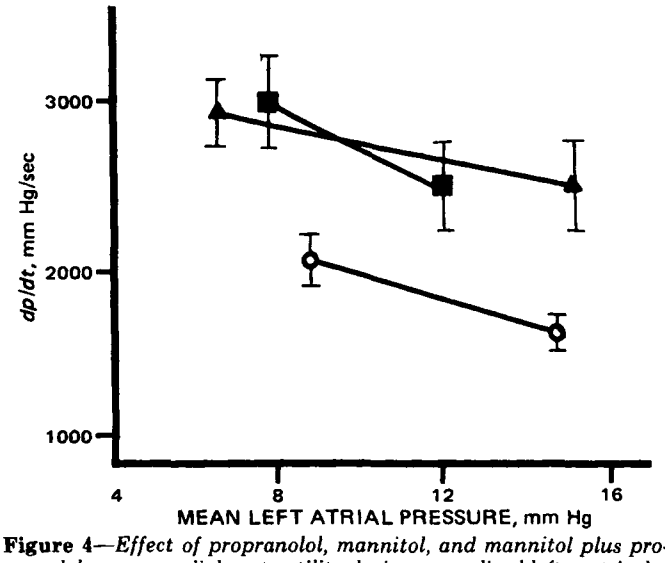

Figure 4—*Effect of propranolol, mannitol, and mannitol plus propranolol on myocardial contractility during generalized left ventricular ischemia. The x axis shows the mean left atrial pressure, and the y axis shows the maximal rate of left ventricular pressure development* (dp/dt). *Key:* ○, *ischemia plus propranolol* ($p < 0.005$); ▲, *ischemia plus mannitol* ($p < 0.025$); *and* ■, *ischemia plus propranolol plus mannitol* ($p < 0.025$).

Table IV—Effect of Propranolol, Mannitol, and Propranolol plus Mannitol during Generalized Left Ventricular Ischemia on Tension–Time Index [a]

Parameter	Control Ischemia	Ischemia plus Drug	Change, %	Level of Significance [b]	Postcontrol Ischemia
		Propranolol			
Tension–time index per beat, mm Hg sec	23.8 ± 0.5	27.2 ± 0.8	14.2 ± 2.1	**	—
Tension–time index per minute mm Hg sec/min	2953 ± 145	2738 ± 161	−7.3 ± 1.3	NS	—
		Mannitol			
Tension–time index per beat, mm Hg sec	24.7 ± 0.9	21.7 ± 0.9	−11.9 ± 0.6	**	24.6 ± 1.0
Tension–time index per minute, mm Hg sec/min	3163 ± 171	2862 ± 182	−9.7 ± 1.2	*	3192 ± 191
		Propranolol plus Mannitol			
Tension–time index per beat, mm Hg sec	24.1 ± 0.9	20.6 ± 0.7	−13.9 ± 1.1	**	23.9 ± 0.8
Tension–time index per minute mm Hg sec/min	3075 ± 177	2258 ± 146	−26.7 ± 1.2	**	3077 ± 173

[a] Each value is expressed as the mean ± *SEM* of six experiments. [b] *, $p < 0.05$; **, $p < 0.001$; and NS, not significant ($p > 0.05$).

centration in a superficial area of the muscle fiber (21). In contrast, Apstein *et al.* (22) were unable to observe any increase in contractility in the isolated hypoxic tissue by mannitol. However, this discrepancy may be explained by the fact that Apstein *et al.* used smaller concentrations of mannitol than those used by the other investigator.

The present study shows that hypertonic mannitol enhances myocardial contractility in the ischemic failing heart without raising myocardial oxygen demands. Although the mean arterial oxygen levels were not measured directly in this investigation, the changes in the tension–time index reflected alterations in the myocardial oxygen requirements (13). These observations were consistent with the findings of Sarnoff *et al.* (23), who showed that norepinephrine may exert a positive inotropic effect in the ischemic failing heart without augmenting mean arterial oxygen levels; the inotropic action of norepinephrine was associated with a marked decrease in the left ventricular end diastolic volume (23). This effect, according to the Laplace principle, tends to lower myocardial tension and offsets the rise in the mean arterial oxygen levels produced by increased contractility. A similar effect also was demonstrated in the pharmacologically depressed dog after ouabain administration (24).

Although the decrease in ventricular tension may prevent a rise in the mean arterial oxygen levels associated with augmentation of myocardial contractility, the present results suggest that the systolic ejection time also may be an important factor. Figure 4 and Tables III and IV show that mannitol augmented myocardial contractility whereas the heart rate and the mean systemic arterial pressure were not altered significantly. As contractility rose and the systolic ejection time decreased, the tension–time index per beat fell. The fact that the tension–time index per minute was lowered significantly, although neither the heart rate nor the mean systolic pressure was decreased, indicates that augmentation of myocardial contractility may diminish myocardial oxygen requirements in the failing heart by decreasing the systolic ejection time.

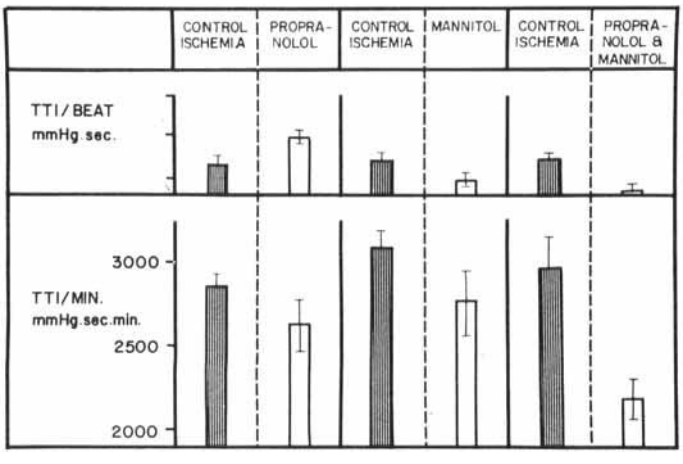

Figure 5—*Effect of propranolol, mannitol, and propranolol plus mannitol on the tension–time index during generalized ventricular ischemia. The* y *axis shows the tension–time index per beat (TTI/beat); the* x *axis shows the tension–time index per minute (TTI/min).*

In contrast, propranolol, through reduced contractility (Fig. 4) and a lowered heart rate (Table III), produced no significant decrease in myocardial oxygen requirements. Figure 5 demonstrates the effects of propranolol on the tension–time index; the tension–time index per minute was not altered significantly whereas the tension–time index per beat exhibited a pronounced increase. The results show that propranolol raised the myocardial oxygen demands by increasing the systolic ejection time (Table III), thereby offsetting the oxygen-sparing effect of its negative chronotropic action. This observation suggests that the principal benefit of β-adrenergic blocking drugs such as propranolol lies in their ability to lower the heart rate rather than to reduce myocardial contractility. Consistent with this view are the findings of Balcon (25), who showed that propranolol could not reduce the pain threshold in patients in whom angina was produced by electrical pacing of the heart. Evidently, the failure of the drug to exert its negative chronotropic activity prevented its beneficial effect. The most pronounced decreases in myocardial oxygen requirements were noted in this study when the myocardial depressant action of propranolol was prevented by administration of hypertonic mannitol (Fig. 5).

Furthermore, when the mean systolic pressure was multiplied by the heart rate, there was a significant drop in the tension–time index per minute after the combined administration of mannitol with propranolol (Fig. 5). The fall in this product was much greater than that achieved by mannitol or propranolol alone. This result was due largely to the fact that propranolol lowered the heart rate whereas the systolic ejection times and the mean systolic pressures, as compared to mannitol, were not very different.

The findings of the present investigation suggest that augmentation of contractility in the ischemic failing heart by a positive nonadrenergic agent may potentiate the beneficial effects of propranolol on the myocardial oxygen balance and prevent cardiac depression. Mannitol is an inotropic agent with highly desirable properties since it does not produce any unwanted hemodynamic or ECG changes. Finally, the ability of mannitol to augment myocardial perfusion (9) may be of great physiological importance. Although further studies are required, augmentation of myocardial contractility and the reduction in heart rate brought about by the combined action of mannitol and propranolol may prove to be an effective therapeutic modality in the control of acute irreversible ischemia.

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Synthesis and Cyclization of Dialkylmalonuric Esters

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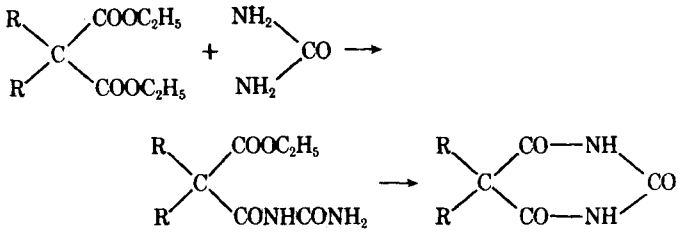
Abstract □ A novel method for the synthesis of methyl dialkylmalonuric esters was developed using the base-catalyzed ring opening of an isopropylidene malonic ester with urea as the key step. The rates of cyclization of these malonuric esters to the corresponding barbituric acids then were studied at buffer concentrations ranging from 0.01 to 1.00 *M*. The reaction was shown to be general base catalyzed, and the reaction rate was found to be subject to a deuterium isotope effect, $k_{H_2O}/k_{D_2O} = 1.3$. The thermodynamic activation parameters also were determined. A three-step mechanism for the conversion of malonuric esters to barbituric acids was proposed; it involved a rapid cyclization step, followed by proton removal by a general base catalyst and a rate-determining collapse of the resulting tetrahedral intermediate aided by a general acid.

Keyphrases □ Dialkylmalonuric esters—synthesis, cyclization to barbituric acids □ Barbituric acids—mechanism for formation by cyclization of malonuric acids □ Activation parameters—cyclization of dialkylmalonuric esters to barbituric acids

Numerous barbituric acids have been synthesized over the past 75 years, but the mechanism of the reaction has not been defined. Barbituric acids usually are prepared by reacting a malonic ester with urea in the presence of a basic catalyst. The mechanism proposed some years ago (1) involved the formation of an intermediate malonuric ester (I), followed by rapid ring closure to the barbituric acid product (Scheme I).

The fact that malonuric esters undergo facile cyclization in basic media was suggested in 1907 (2), and later these esters were shown to cyclize rapidly in weakly basic solution (1). In a continuing effort to elucidate the mechanism of the reaction between malonic esters and urea, the present study focuses on the cyclization step.



I

Scheme I

BACKGROUND

Although no kinetic studies on the cyclization of malonuric esters have been reported, hydrolysis of barbituric acids in alkaline solution has been investigated. Such hydrolyses produce various products, including the malonuric acid. A complete scheme for the alkaline decomposition of barbiturates was given by Aspelund (3), and a kinetic study on the hydrolysis rate of barbituric acid derivatives was reported by Garrett *et al.* (4). The latter report stated that malonuric acids are capable of hydrolysis to the barbituric acids at pH 7–10, and the enthalpy (ΔH^*) and entropy (ΔS^*) of activation were determined for the hydrolysis of numerous barbituric acids.

One facet of the present investigation involved determination of the mechanism of base catalysis in the cyclization of esters of diethylmalonuric acid. The classical experiment for distinguishing general base catalysis from specific base catalysis involves determining the reaction rate in a series of buffers of constant pH but with varying concentrations of total buffer species at constant ionic strength (5). General base catalysis, which requires catalysis by all bases present, is demonstrated if the reaction rate depends on the total absolute buffer concentration. Specific base catalysis is independent of the absolute buffer concentration.

UV spectrophotometry was employed to monitor the cyclization reaction of the malonuric esters. A pH 10 buffer system was chosen since at that pH the barbituric acid product exists as the monoanionic species (99+%), which absorbs strongly at 240 nm (6), facilitating easy assessment of its formation rate.

This method also was used to measure the reaction rates in deuterium oxide since a comparison of rates in heavy *versus* light water can be valuable in mechanism elucidation. Jencks (7) reported that because of zero-point energy differences and different stretching frequencies, rate constants for OH and OD bond cleavage can be 10-fold larger for hydrogen than for deuterium.

Determination of the thermodynamic activation parameters also was undertaken. The entropy and enthalpy of activation were obtained from plots of $\log k/T$ *versus* $1/T$ (8).

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