Further Studies of the Effects on Myocardial Energy Utilization Elicited by Nitroglycerin

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Since sympathetic stimulation could play an important part in the short term attacks of angina pectoris, the effects of nitroglycerin on the myocardial response to levarterenol were investigated. Levarterenol infusions directly increased the isometric systolic tension (IST) developed by the cardiac muscle. Nitroglycerin had no apparent direct effect on this increase in IST either in the normal heart or in the heart following partial coronary ligation. In experiments in which IST was measured with a lever system adjusted to the end-diastolic length and tension occurring just prior to the contraction, nitroglycerin was found to have an indirect depressant effect on the levarterenol response. This decrease in contractility was shown to be secondary to a decrease in hemodynamic work load elicited by nitroglycerin. It is suggested that the chief effect of nitroglycerin in the relief of angina pectoris is due to this agent's ability to decrease a work load imposed on the myocardium by sympathetic stimulation.

The effectiveness of nitroglycerin in the relief of angina pectoris has been largely interpreted in terms of the ability of this agent to dilate the coronary vessels. However, Gorlin and coworkers have reported that nitroglycerin does not change coronary vascular resistance in patients with severe heart disease. In these patients nitroglycerin did not affect myocardial oxygen consumption or coronary flow, but the cardiac work decreased resulting in a reduction in cardiac efficiency. These and other investigators suggested the possibility of an altered energy metabolism by nitroglycerin. A number of investigators have presented evidence which indicates an increased sympatho-adrenal function during periods of angina pectoris. Raab suggested that nitroglycerin interfered with the metabolic anoxia-producing effects of the sympathomimetic amines. Eckstein and co-workers concluded that nitroglycerin did not alter the adrenergic-produced increase in oxygen consumption, in rate, or in estimated systolic vigor of the normal dog's heart. Olson and Piatnek have suggested that myocardial metabolism might be divided into 3 general phases: 1) energy liberation, 2) energy conservation, and 3) energy utilization. It is possible sympathetic stimulation may affect any one or all 3 such phases. Further, nitroglycerin could alter the effects of sympathetic stimulation on any of these possible metabolic phases.

In a previous series of experiments nitroglycerin was found to be without a direct effect on isometric systolic tension (IST) measured at a fixed initial tension and fixed initial length. The hypotension elicited by nitroglycerin provoked an increase in ventricular contractile force which was shown to be due to sympatho-adrenal stimulation. These results do not support the possibility that nitroglycerin has a direct metabolic effect on the energy utilization phase of metabolism in the normal dog heart since the isometric tension developed by the muscle contraction indicates the maximum working capacity of the muscle and depends primarily on the change in free energy accompanying the contraction. In the present investigation the effects of nitroglycerin were studied on the increase in IST elicited by levarterenol stimulation. These effects were determined before and after partial coronary ligation. In addition, an evaluation was made of the effects

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of changes in heart size on the IST response elicited by levarterenol alone and by the administration of nitroglycerin during the levarterenol response. The data derived from these studies indicate that while nitroglycerin has no apparent direct effect on the amount of free energy released with each contraction of the myocardium during the levarterenol response either before or after partial coronary occlusion, nitroglycerin did decrease the increment in IST elicited by levarterenol. This decrease in IST was secondary to a decrease in heart size caused by a decrease in left atrial filling pressure.

Methods

Studies of the influence of levarterenol on IST and of nitroglycerin on the levarterenol response were conducted in 35 open-chest mongrel dogs. In all experiments, anesthesia was obtained with 10 mg./Kg. morphine subcutaneously, followed in 30 min. by 15 mg./Kg. pentobarbital intravenously. A metal tube was passed through the left carotid artery into the ascending aorta. A Statham transducer (P 23-D) attached to the metal tubing was used to determine aortic blood pressure. A Sanborn Polyviso Model 154 was used for amplification and recording of blood pressure, IST, and electrocardiograms. Indwelling polyethylene catheters were passed from both the right and left femoral veins into the inferior vena cavae to approximately the level of the diaphragm. A tracheotomy was performed and artificial respiration was provided by a rhythmic, motor driven interrupter connected between the animal and a constant positive pressure air supply. Prior to mid-line thoracotomy an additional injection of 5 mg./Kg. pentobarbital was administered intravenously.

In 25 experiments IST was recorded by the strain gage arch method. Detailed descriptions of this method for measuring IST have been published.\textsuperscript{12-14} The strain gage arch was attached to the wall of the left ventricle with cotton sutures. The sutures were tied approximately 1 cm. above the apex and 0.5 cm. below the left marginal arteries, perpendicular to and about 0.8 cm. below the anterior descending branch of the left coronary artery. The muscle segment between the two points of attachment was stretched approximately 50 per cent of its initial end-diastolic length. This stretch produced an increase in the initial tension of approximately 75 to 100 Gm. above end-diastolic tension. Such measurements of IST have been shown to be primarily influenced by humoral substances such as hormones, drugs, and oxygen supply, or by changes in neurogenic tone, essentially that of the sympathetic system. An infusion of levarterenol was started in 19 of the 25 animals. In 17 of the animals receiving this amine, nitroglycerin in a dose of 0.1 mg./Kg. was administered after a stable recording period of 5 min. In the other 2 animals ligation of the descending branch of the left coronary artery was accomplished and then nitroglycerin was administered. In 4 of the 17 animals receiving both levarterenol and nitroglycerin, the levarterenol infusion was discontinued. After 30 min. the coronary artery was ligated. Following a 15 min. period of stable recordings a second infusion of levarterenol was started. Nitroglycerin was again administered during the levarterenol infusion. In 6 of the 25 animals ligation of the coronary artery was completed before administration of either of these agents.

In 10 of the 35 experiments IST was measured with the strain gage lever system.\textsuperscript{15} The 2 feet of the strain gage lever system were attached to the left ventricle in the same manner as that described for the attachment of the feet of the strain gage arch. However, the length of the muscle segment between the 2 points of attachment followed the changes in end-diastolic heart size and the lever system was adjusted accordingly so that the increase in isometric tension developed with each systole was measured at the end-diastolic length occurring just prior to the contraction. Mid-cervical vagosympathectomy and preganglionic sympathetic blockade were performed initially in 4 of the 10 experiments utilizing the lever system. The remaining 6 experiments were conducted in dogs with intact cardiac nerve supply—3 experiments in animals with controlled left atrial pressure and 3 with intact circulation.

In the 12 instances of coronary ligation, partial occlusion of the descending branch of the left coronary artery just distal to the left circumflex branch was accomplished by ligation against a 15 gage needle. The needle was then removed. The left ventricle became cyanotic and dilated, and electrocardiographic changes developed which were typical of myocardial ischemia. This procedure is expected to decrease the blood supply to the area of the myocardium to which the strain gage arch was attached.

Preganglionic sympathetic blockade was obtained according to the technic described by Brewster et al.\textsuperscript{16} except tetracaine in a solution of 0.05 per cent was used in place of procaine. The tetracaine was delivered into the epidural space through indwelling polyethylene tubes placed about the level of the seventh cervical, fifth thoracic, and second sacral vertebrae, respectively. Mid-cervical
Table 1
Effects of Levarterenol Infusion and Nitroglycerin on the Levarterenol Response. Isometric Tension Measured at a Fixed Initial Length and Initial Tension.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of Exper.</th>
<th>Per cent change in Isometric Contractile Force (Mean)</th>
<th>Per cent change in Diastolic Pressure (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levarterenol Infusion</td>
<td>19</td>
<td>75 S.E.±12.0</td>
<td>94 S.E.±9.9</td>
</tr>
<tr>
<td>Nitroglycerin during Levarterenol Infusion</td>
<td>17</td>
<td>17 - 8.9 ± 2.7</td>
<td>-46 ± 3.7</td>
</tr>
<tr>
<td>Levarterenol Infusion after Occlusion</td>
<td>8</td>
<td>45 S.E.±18.5</td>
<td>104 S.E.±26.4</td>
</tr>
<tr>
<td>Nitroglycerin during Levarterenol Infusion after Occlusion</td>
<td>10</td>
<td>1 ± 7.9</td>
<td>-47 ± 4.1</td>
</tr>
</tbody>
</table>

Vagotomy preceded sympathetic blockade in each of these experiments.

Left atrial pressure was controlled in 3 experiments. A Bardic 20 catheter was placed in the left atrium through the left auricular appendage. This catheter was connected to a polyethylene tube (8 mm. inside diameter) which was in turn connected to the lower arm of a "Y" tube. A modified Mariotte bottle was attached to the upper arm of the "Y" tube. The pressure in the Mariotte bottle was adjusted so that left atrial filling pressure would remain at control levels of approximately 6 cm. of blood. The other upper arm of the tube was left open to the atmosphere at a point approximately 6 cm. above the left atrium. This procedure has been found to maintain left atrial pressure within 0.5 cm. of water.

Commercial forms of levarterenol and nitroglycerin were used (Levophed, Winthrop and nitroglycerin, Lilly). The infusion of levarterenol was administered through the venous catheter in the left leg and the nitroglycerin through the venous catheter in the right leg. This permitted administration of nitroglycerin without disturbing the infusion of levarterenol.

Results
Measurements of Isometric Systolic Tension at a Fixed Initial Length and Tension (Strain Gage Arch)

In the experiments summarized in table 1 levarterenol was administered by intravenous infusion at a rate sufficient to increase diastolic pressure approximately 100 per cent. Figure 1 illustrates the typical effects of levarterenol infusion and the status of the response elicited by nitroglycerin in the normal heart and in the heart with partial coronary ligation.

The levarterenol was started prior to coronary ligation in 19 dogs. The average increase in IST elicited was 75 per cent above control levels. Sinus bradycardia developed with the increase in arterial pressure. Frequent, short intervals (2 to 5 beats) of A-V nodal tachycardia occurred. Such intervals were apparently associated with ventilation of the lungs. In 17 of these animals a constant rate of infusion was established and a stable recording period of 6 min. was obtained. Nitroglycerin (0.1 mg./Kg.) was injected. During the elicited decrease in diastolic pressure there was an increase in heart rate. No significant change occurred in the force of ventricular contraction. The time interval of the decrease in arterial blood pressure and correction of sinus bradycardia ranged from 5 to 15 min.

Partial Coronary Ligation

Ligation of the descending branch of the left coronary artery was accomplished in 12 dogs. The procedure was completed before administration of either agent in 6 experiments. Two of these animals showed a marked decrease in ventricular contractile force, then developed a short period of alternating beats of high and low amplitude followed by ventricular fibrillation and death. In the animals surviving coronary ligation the ventricular contractile force was only minimally depressed from pre-ligation levels (not greater than 20 per cent). In these animals the average diastolic blood pressure was slightly, though not significantly, lower after coronary ligation.

In all 10 animals with partial coronary ligation the infusion rate of levarterenol was adjusted so that the increment in diastolic pressure was similar to that obtained in the normal animals. At this infusion rate, the increment in IST (per cent of control) was...
significantly less than that seen in the animals prior to coronary ligation. Nitroglycerin had no effect on the increased IST elicited by levarterenol. The mean relative grade of hypotension was similar to that seen prior to coronary ligation (-47 per cent). The increase in ventricular premature contractions, the appearance of pulsus alternans, and the decreased responsiveness to levarterenol in these animals could be due to an increased level of sympatho-adrenal activity caused by the partial coronary ligation.

Measurement of IST Developed at the End-Diastolic Length Occurring Just Prior to Contraction (Strain Gage Lever System)

Ten experiments were carried out using a modified Cushny lever system described by Cotten and Maling. The upper pair of recordings in figure 2 illustrate the measured changes in IST occurring with changes in end-diastolic tension. Left atrial filling pressure was increased from 5 cm. of water to 7, 9, 11, and 15 cm. of water respectively. The corresponding muscle segment lengths were 22 mm. at 5 cm. of water and 22.5, 23, 23.5, and 25 mm. at the other pressure levels. With these increments in muscle segment length the IST increased from 50 µm. at 5 cm. of water to 73 µm. at 15 cm. of water. Similar results have been reported by Cotten and Maling for experiments conducted with the heart-lung preparation. These changes in IST are considered to be secondary to changes in end-diastolic tension, although intrinsic changes due to variations in neuro-humoral influences would be simultaneously determined by the lever system.

 Interruption of Cardiac Nerve Supply

In 4 experiments using the lever system, mid-cervical vagotomy and preganglionic sympathetic blockade were carried out. Vagotomy had little effect on ventricular contractile force or blood pressure. This procedure increased heart rate from control levels of near 110 beats/min. to approximately 180 beats/min. Subsequent preganglionic sympathetic blockade decreased heart rate to about 50 beats/min. This procedure decreased arterial pressure from control levels of approximately 100/70 mm. Hg to levels near 45/25 mm. Hg. The average change in contractile force was a reduction of 80 per cent. The levarterenol infusion was sufficient to increase diastolic pressure to approximately 90 mm. Hg. At this infusion rate ventricular contractile force was approximately 20 per cent above pre-blockade levels. Heart rate was 120 beats/min. There was no apparent change in heart size. Nitroglycerin (0.03 mg./Kg.)
administered during the levarterenol infusion decreased arterial pressure by an average of 50 per cent. The mean decrease in heart contractile force was 35 per cent with no significant alteration of heart rate. The duration of the response to nitroglycerin even in the decreased dose level was significantly longer than the response to 0.10 mg./Kg. in the dog with intact vagus and sympathetic nerve supply. The lower tracing in figure 2 illustrates the decrease in diastolic muscle segment length and IST secondary to the decrease in hemodynamic work load. As the work load increased the muscle segment returned to its pre-injection length. The time interval between the heavy vertical lines is 20 sec.
hemodynamic work load elicited by nitroglycerin during the levarterenol response. The initial length of the muscle segment was adjusted from the control length of 24.0 mm to 23.6, 23.2, and 22.7 mm, respectively. Each reduction in initial length was accompanied by a decrease in IST. Such adjustments were dictated by the decrease in diastolic length of the muscle segment which occurred concomitantly with the reduction in hemodynamic work load. As arterial pressure increased the muscle segment returned to its pre-injection length. Partial occlusion of the thoracic aorta prevented the decrease in ventricular contractile force elicited by nitroglycerin. When the aortic constriction was released during the usual period of hypotension, there was an immediate decrease in arterial pressure followed by a decrease in ventricular force. A second constriction raised arterial pressure while ventricular contractile force increased immediately to near pre-nitroglycerin control levels. Constriction of the inferior vena cavae in 2 of these animals decreased arterial pressure by 20 mm Hg; this procedure also immediately decreased ventricular contractile force by nearly 15 per cent. As arterial pressure decreased with continued partial occlusion there was a concomitant decrease in ventricular contractile force. The decrease in IST elicited by the hypotension secondary to a decrease in venous return was closely similar to the decrease in contractility elicited by nitroglycerin.

Intact Nerve Supply and Circulation

In 3 experiments using the modified Cushny lever system, an evaluation was made of the heart size changes which occurred in animals with intact cardiac nerve supply. The increase in ventricular contractile force elicited by levarterenol was significantly greater than that occurring with only an increase in muscle fiber length. With the administration of nitroglycerin during the interval of levarterenol stimulation, there was a decrease in end-diastolic length as diastolic pressure decreased. A corresponding decrease of nearly 25 per cent occurred in ventricular contractile force. An increase in heart rate was observed during the interval of hypotension. The decrease in diastolic fiber length which occurred with the reduction in cardiac work load was probably responsible for the decrease in IST. These results would seem to emphasize the importance of the Starling's law changes in heart force during the action of nitroglycerin.

Experiments with Controlled Left Atrial Pressure

In 3 additional experiments conducted with the lever system, left atrial filling pressure was controlled. During the hypertension and bradycardia elicited by levarterenol infusion there was a loss of fluid from the left atrium. IST was increased by an average of 75 per cent above control levels. Diastolic fiber length did not change during the levarterenol response. There was no change in the initial diastolic length of the muscle segment during the nitroglycerin response, and correspondingly there was no change in increased IST elicited by levarterenol. Nitroglycerin decreased arterial pressure in spite of the fact that left atrial pressure did not decrease and there was an apparent increased stroke volume as estimated from the contour of the aortic pressure pulse.

Discussion

As Szent-Gyorgyi points out, the contractile matter of the heart is embedded in well developed oxidative machinery.\textsuperscript{11} However, when the oxygen supply to the muscle becomes inadequate due to increased demands on the work capacity of the muscle or by obstruction of the oxygen supply, the heart must rely on the stored high energy phosphates and its glycolytic system. The recent work of Katz et al. would indicate that the catechol amines directly affect metabolism and energy release by cardiac muscle.\textsuperscript{5, 17, 18} Brewster and associates,\textsuperscript{19} using strain gage arches for measurements of isometric tension, have shown that relaxation has the kinetic characteristics of an enzymatic reaction associated with and dependent upon chemical bond energy or electron transfer. These investigators further state that an inverse exponential
relationship existed between the metabolic rate and the duration of the contracted state as well as the time required for relaxation. In the present investigation levarterenol was found to increase the IST developed by both the normal and ischemic heart. Such changes would indicate an increase in the release of free energy with each contraction of the myocardium. Nitroglycerin was found to be without a direct effect on this increased release of free energy. However, the change in free energy and the rate of the reaction are not related. While these and other experiments\textsuperscript{10} show that nitroglycerin has no direct effect on the free energy changes occurring during normal myocardial contraction or contraction stimulated by levarterenol, there is no evidence presented which indicates that nitroglycerin does not have an effect on the rate of release or build up of chemical energy.

The following possible conclusions are supported by the observations made in the experiments in which ventricular contractile force was measured at the end-diastolic length and tension just prior to the contraction. In the intact animal, levarterenol increases not only IST by direct myocardial stimulation, but also end-diastolic heart size during the period of hypertension and associated bradycardia. The resulting increase in end-diastolic fiber length indirectly increased the force of cardiac contraction but to a much lesser degree than the force increase due to direct myocardial stimulation. It is well known that there is an increase in oxygen consumption accompanying an increase in the force of cardiac contraction resulting from an increase in hemodynamic work load.\textsuperscript{17, 21} Nitroglycerin therapy markedly reduces the hemodynamic work load and thus reduces the oxidative energy required to do pressure-volume work.

In the experiments in which coronary blood supply to the muscle segment was reduced by coronary ligation the oxygen supply to the area would correspondingly be reduced. The reduction in blood supply and available oxygen could lead to an increase in acid metabolites of anaerobic metabolism, and these could be further increased by the metabolic effects of levarterenol. The effects of nitroglycerin in these animals theoretically could increase coronary flow and facilitate the removal of these metabolites. The lack of an effect by nitroglycerin on myocardial contractility either before or during levarterenol infusion in the animals following partial coronary occlusion supports the belief that the major effects of nitroglycerin in the relief of angina pectoris are extra-coronary.

In the patient with angina pectoris there is good evidence that the sympathetic nervous system is called upon during the periods of increased demands on the heart. Levine\textsuperscript{6} reports that in patients with pain associated with angina pectoris the blood pressure will rise almost invariably. Studies now in progress here show that this is especially true of the diastolic pressure. Gazes and associates\textsuperscript{7} have reported increased plasma levels of the catechol amines occurring with angina pectoris. From the present experiments it would seem that the major action of nitroglycerin in the relief of angina pectoris is due to its ability to reduce this increased hemodynamic work load on the myocardium. Gorlin and associates\textsuperscript{22} have reported that the coronary blood flow is essentially fixed in patients with severe heart disease. Further, these investigators have shown that the myocardial oxygen consumption related to the pressure-volume work done is actually greater following nitroglycerin. Based on these observations they concluded that the clinical effects elicited by nitroglycerin were largely due to a decrease in the contractility of the heart. The present investigation supports this conclusion and indicates that the decrease in contractility is primarily due to the decrease in hemodynamic work load rather than to a direct effect of nitroglycerin on intrinsic myocardial contractility. The work of Brachfeld and co-workers\textsuperscript{23} and Johnson and associates\textsuperscript{24} show that in man, nitroglycerin decreases pulmonary pressure and right atrial pressures as well as arterial pressure. These findings add...
additional support to the conclusion that the important effects of nitroglycerin on cardiac contractility are largely elicited through a reduction in contractility in accordance with Starling's law.

Summary
Nitroglycerin had no effect on the increased force of contraction (measured with the strain gage arch) elicited by levaterenol. While partial coronary occlusion decreased IST the drug responses obtained were essentially the same as those which occurred in the normal animal. These findings indicate that nitroglycerin has no effect on the increased amount of free energy released with each contraction or the maximum working capacity of the myocardium under these conditions. In the experiments in which IST was measured with a lever system adjusted to the end-diastolic length and tension occurring just prior to the contraction, nitroglycerin decreased myocardial contractility. This decrease in contractile force was shown to be secondary to the decrease in hemodynamic work load. It is suggested that the chief effect of nitroglycerin in the relief of angina pectoris is due to this agent's ability to decrease a work load on the myocardium imposed by sympathetic stimulation.

Summario in Interlingua
Nitroglycerina exerceva nullo effetto super le augmentata forta de contraction (measurata per medio del alterubi describite metodo del arco de tensioniometro 12-14) que esseva evocate per levaterenol. Durante que partial occlusion coronari reduceva le isometric tension systolica, le responsa evocate per le droga esseva essentially le mesmes como illos observe in le animal normal. Iste constatationes indica que nitroglycerina ha nullo effetto super le augmentata quantitate de energia libere che deveni disponibile con omne contraction individual o super le maximo del capacitate de labor del myocardio sub iste condiziones. In le experimentos in que le isometric tension systolica esseva measurata per un systema de levarores ajustate al longor e al tension termino-diastolica ocurrente justo ante le contraction, nitroglycerina reduceva le contractilitate myocardial. Esseva demonstrate que iste reduction del forta de contraction esseva un occurrentia secundari al reduction del carga de labor hemodynamic. Es presente le these que le major effetto de nitroglycerina in le allevamento de


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