14. Feinberg H, Katz LN: Effect of catecholamines, /-epinephrine and /-


Cholinergic Intervention on Myocardial Dynamics and Metabolism in the Nonworking Dog Heart

VINCENT V. GLAVIANO, JACK M. GOLDBERG, MARIE PINDOK, DON WALLICK, AND CHRIST ARAVANIS

SUMMARY In electrically paced hearts of dogs subjected to cardiopulmonary bypass, intracoronary infusion of carbamylcholine chloride (carbachol; 1 /g/kg per min) caused, in the absence of external cardiac work, an increase in coronary blood flow (46%) and a decrease in both myocardial force of contraction (53%) and systemic perfusion pressure (20%). Pretreatment with atropine effectively blocked these responses to carbachol. Previous treatment with reserpine indicated that adrenergic and cholinergic interactions did occur, as observed from the more significant changes in these parameters in the absence of catecholamines. Compared with control, carbachol significantly lowered both myocardial extraction ratios (50-25%) and uptake of oxygen (4.2-3.1 ml/min per 100 g). The decrease in oxygen metabolism accompanied a significant increase in both myocardial extraction (6-18%) and myocardial uptake (1.3-5.2 mg/min per 100 g) of plasma triglycerides. The increase in triglyceride uptake was accompanied by a decline in intracellular triglyceride hydrolysis, as verified by more than a 2-fold increase in percent of 3H-sodium palmitate substrate. While triglyceride uptake increased partly because of an increase in coronary blood flow, it was postulated that carbachol decreased myocardial cyclic adenosine 3',5'-monophosphate (cyclic AMP) by a mechanism similar to that previously reported for acetylcholine and prostaglandin E1. The inhibition of intracellular lipolysis by cholinergic intervention elevates myocardial triglyceride levels, a response that is opposite to that reported for myocardial sympathetic stimulation.

THE EFFECTS OF cholinergic substances on myocardial metabolism have been investigated extensively since Szekeres et al.1 reported that acetylcholine (ACh) prevented a fall in cardiac muscle stores of glycogen in rats exposed to hypoxia. Since this work was reported, ACh has been shown to reduce formation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in panicle preparations of adenylate cyclase from dog hearts.2 This study was followed by the observation that ACh caused a rise of cyclic guanosine 3',5'-monophosphate (cyclic GMP) in the isolated perfused rat heart.3 One of the most significant actions of the cholinergic neurotransmitter has been its regulation of cardiac dynamics and metabolism through its interaction with adrenergic substances in heart muscle.4,5 Blukoo-Allotey et al.4 showed cholinergic-adrenergic interaction when they found that simultaneous administration of epinephrine and ACh inhibited glycogenolysis by epinephrine. Glaviano et al.5 reported that ACh antagonism of norepinephrine stimulation of lipid uptake and utilization in the intact dog heart was mediated through
inhibition of adenylate cyclase activity.

The action of cholinergic substances on cardiac metabolism has been difficult to analyze because of the influence that a change in external cardiac work would have on cardiac consumption of metabolic substrates. Experiments were therefore undertaken to use the cardiopulmonary bypass preparation in the dog to obtain a cholinergic response to intracoronary administration of the choline ester, carbamyl-choline chloride (carbachol) in the absence of a change in cardiac work. Aside from its action as a cholinergic agent, the agonist was used in preference to ACh because of its total resistance to hydrolysis by acetylcholinesterase and nonspecific cholinesterase. The focus of the experiments centered on determining the effects of carbachol on coronary blood flow and myocardial uptake of oxygen, free fatty acids (FFA), and triglycerides in the normal heart and in hearts pretreated with the muscarinic-blocking drug atropine or with the catecholamine-depleting drug reserpine.

Methods

Twenty-eight male mongrel dogs averaging 18 kg in weight were anesthetized with sodium pentobarbital (30 mg/kg, iv). The trachea was intubated, and positive-pressure ventilation was provided with a Harvard pump. A midsternal thoracotomy was performed, and the heart was suspended in a pericardial cradle. After administration of sodium heparin (3 mg/kg), the dog was placed on a cardiopulmonary bypass system that had been primed with 700–800 ml of Krebs-Ringer bicarbonate solution and 500 ml of blood from a donor dog anesthetized with sodium pentobarbital. Venous blood drained from the superior and inferior venae cavae was allowed to enter a 2-liter Travenol oxygenator bag receiving a fine stream of 100% O₂. Blood from the oxygenator was returned to the dog by means of a calibrated Sarns portable roller pump after passing through a glass coil immersed in a water bath maintained at 39°C. From the heat exchanger, blood entered a cannulated right femoral artery and a cannulated left subclavian artery close to the arch of the aorta for perfusion of the coronary arteries. The cannulated left subclavian artery supplied blood only to the coronary arteries, since the aorta was clamped just distal to the left common carotid artery for perfusion of the trunk and head region, respectively. A cannula was inserted into the left subclavian artery close to the arch of the aorta for perfusion of the coronary arteries. The cannulated left subclavian artery supplied blood only to the coronary arteries, since the aorta was clamped just distal to the artery and the brachiocephalic artery was tied close to its origin from the aortic arch. To monitor total coronary inflow, a model EP 300-3/16 calibrated external flow probe was connected to a Carolina Medical Electronics flowmeter (model 501), interposed between the subclavian artery and the perfusion system. The oscillations imparted to the perfusion fluid by the Sarns pump were partly damped by an air chamber added to the coronary perfusion circuit. To drain and sample coronary venous blood, an intracardiac sucker sleeve was inserted from the right atrial appendage into the right ventricle. To ensure the complete collection of the coronary venous outflow from the right ventricle, the pulmonary artery was clamped at its origin from the right ventricle. To drain the left ventricular Thebesian flow and any blood escaping through the aortic valve, a drainage tube was inserted into the apex of of the left ventricle and connected to the oxygenator. The volume of blood drained from the left ventricle ranged from 13 to 24 ml/min. Thus, with both right and left ventricles devoid of blood and opened to atmospheric pressure, the effects of preload and afterload on cardiac dynamics were minimal and constant. To record changes in myocardial force of contraction, a Walton-Brodie strain gauge arch was sutured to the myocardium over the conus region of the right ventricle. The hearts were electrically paced with a Travenol model 808 pacemaker connected to a pair of fishhook electrodes inserted into the epicardium of the right ventricle close to the atrial-ventricular groove. The pacemaker was adjusted to maintain heart rate at 10–15 beats/min above the intrinsic rate. Systemic body perfusion pressure and coronary inflow pressures were measured with calibrated Statham P23Db transducers. These two pressure parameters, together with total coronary outflow, right ventricular contractile force, and a limb lead electrocardiogram, were recorded on a direct writing 8-channel Beckman model RM dynograph.

To determine whether a minimal dose of carbachol could stimulate cardiac metabolism in the absence of gross changes in peripheral vascular resistance, the metabolic effects produced by doses of carbachol ranging from 0.5 to 1.2 μg/kg per min were studied in pilot experiments. It was found that administration of 1 μg/kg per min consistently produced an increase in myocardial triglyceride uptake and a decrease in myocardial oxygen uptake. These metabolic changes were found to occur in the absence of an overwhelming decrease in peripheral vascular resistance. A total of 28 dogs were used in this study. In the first group made up of eight dogs, carbachol, (1 μg/kg per min) (Sigma) was infused into the coronary perfusion circuit for 6 minutes. All drug infusions into the coronary circulation were made with a model 940 Harvard constant infusion pump. The second group (six dogs) was infused with carbachol 5 minutes after a blocking dose of atropine sulfate (1 mg/kg) had been administered. To eliminate a possible interaction between the release of norepinephrine by adrenergic nerve endings and carbachol, a third group made up of three dogs was pretreated with reserpine (Serpasil) to deplete cardiac catecholamines. The dogs that were reserpinized received an intramuscular injection of 0.3 mg/kg per day for 2 days, followed by an additional 0.5 mg/kg injected on the day of the experiment after induction of anesthesia. The effectiveness of reserpine treatment in depleting cardiac catecholamines was tested by the absence of a change in heart rate or right ventricular contractile force during supramaximal electrical stimulation of the right stellate ganglion. In a fourth group, consisting of 11 dogs, a radioisotope study was conducted on the cardiac uptake of FFA and triglycerides.

After the dogs had been placed on total cardiopulmonary bypass and stabilized with ventricular pacing for approximately 10 minutes, with the exception of the bypass dogs receiving radioactive isotopes, control blood samples were simultaneously drawn from the femoral artery and right ventricle. At the end of the 6-minute period of carbachol infusion and with the infusion continuing, another set of arterial and coronary venous blood samples was drawn. Blood samples were analyzed for oxygen content, plasma FFA, and plasma triglycerides. The arterial-
coronary venous difference and substrate uptake by the heart were calculated for the control period and at the end of the carbachol infusion. The extraction by the heart of a metabolic substrate from plasma was measured as the myocardial extraction ratio in which, the ratio, expressed as percent, was calculated by dividing the arterial-coro-

nary venous blood difference by the arterial level and multiplying by 100. The extraction ratio would therefore express the percent of the substrate extracted by the heart from the total concentration present in arterial blood enter-
ing the heart. The myocardial uptake of a substrate expressed in milliliters or μEq/min per 100 g of cardiac muscle was determined from the product of the myocardial arterio-coronary venous blood difference and coronary blood flow. Oxygen content of anaerobically drawn blood samples was measured in duplicate by the microgas-

ometric method of Roughton and Scholander. Plasma FFA levels were determined in duplicate samples by titra-

tion according to the method of Dole and Meinetz, as modified by Goss and Lein. Plasma triglycerides were measured with a triglyceride kit (Boehringer Mannheim Corp.) that uses saponification of triglycerides with alco-

holic KOH. The amount of nicotinamide adenine dinu-

cleotide consumed was equated to the amount of glycerol liberated from the triglycerides.

In the radioisotope study, dogs on cardiopulmonary bypass were infused by coronary route simultaneously with 3H-palmitic acid and 14C-tripalmitin. Five mCi of

[9,10-3H]palmitic acid sodium salt was sonified in 25 ml of a 3.7% albumin-0.9% NaCl solution, while 1 mCi of [carboxyl-14C]tripalmitin was sonified in 10 ml of 3.7% albumin-0.9% NaCl solution. The 3H-FFA solution was divided into equal 1-ml portions and the 14C-tripalmitin was divided into equal 0.7-ml portions which were sealed and stored at -20°C. For each experiment, one portion of both 3H-FFA and 14C-tripalmitin was thawed, combined in a single test tube, and brought to a final volume of 8 ml with 0.9% NaCl solution. The radioisotope solution was transferred to a syringe and infused into the coronary circulation at the rate of 1 ml/min for 6 minutes with a constant infusion pump; this represented a total dose of 150 μCi of 3H-FFA and 50 μCi of 14C-tripalmitin. Six of the 11 dogs infused with isotopic substances had already had a 6-minute infusion of carbachol which was allowed to continue until completion of the isotope infusion. At the end of the perfusion period, the entire left ventricular wall was excised and placed in ice-cold Krebs-Ringer bicarbo-

nate solution. The tissue was taken immediately to an ice bath, and coronary vessels were perfused at 90 ml/min. Thirty seconds after the onset of carbachol infusion, there was a gradual decrease in right ventricular force of contraction that was accompanied by a significant increase in coronary blood flow. As the infusion was continued, ventricular force of contraction stabilized at 55% of control, while the initial increase in coronary blood flow to 145 ml/min was followed by a moderate decrease (115 ml/min), although remaining at a level higher than control for the duration of the infusion. The average changes in mean systemic blood pressure, mean coronary perfusion pressure, coronary blood flow, and myocardial force of contraction after 6 minutes of carbachol infusion in eight dogs are summarized in Figure 2. By altering peripheral vascular resistance, carbachol produced a significant decrease in systemic blood pressure (111 ± 4 to 89 ± 5 mm Hg, P < 0.001) that was accompanied by an average decrease in myocardial force of contraction of 53 ± 4% (P < 0.001). Since the perfusion pump output was constant, vasodila-

Figure 1 An original recording representative of the effects of carbachol (1 μg/kg per min) by intracoronary route on systemic and coronary perfusion pressure, right ventricular force, coronary flow, and lead II of the electrocardiogram (ECG). The heart was electrically paced at 200 beats/min.
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![Diagram](https://example.com/diagram.png)

**FIGURE 2** Mean changes in systemic perfusion pressure (SP), coronary perfusion pressure (CPP), coronary blood flow (CBF), and percent change in cardiac force (CF) before and after 6 minutes of intracoronary infusion of carbachol in eight dogs subjected to a cardiopulmonary bypass. Vertical line on top of bars indicates the standard error of the mean.

Atropine (1 mg/kg) was administered 5 minutes before carbachol infusion; n= eight dogs.

**TABLE 1** Alterations in Myocardial Extraction Ratio (ER) and Uptake (U) of Oxygen, Free Fatty Acids (FFA), and Triglycerides

<table>
<thead>
<tr>
<th></th>
<th>Oxygen (ml/100 ml)</th>
<th>FFA (µEq/liter)</th>
<th>Triglycerides (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbachol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50 ± 2</td>
<td>4.2 ± 0.3</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Carbachol</td>
<td>25 ± 1*</td>
<td>3.1 ± 0.2*</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Atropine</td>
<td>45 ± 3</td>
<td>4.5 ± 0.4</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Atropine +</td>
<td>47 ± 5</td>
<td>4.9 ± 0.4</td>
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</tr>
<tr>
<td></td>
<td>48 ± 4</td>
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Results are expressed as mean ± SE.

Carbachol (carbamylcholine chloride) was infused into the coronary arteries at the rate of 1 µg/kg per min for 6 minutes; n = eight dogs.

**TABLE 2** Arterial Levels of Oxygen, Plasma Free Fatty Acids (FFA), and Triglyceride in Response to Carbamylcholine Chloride (Carbachol) before and after Muscarinic Blockade with Atropine

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<tr>
<td>Control</td>
<td>15.9 ± 0.4</td>
<td>499 ± 30</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>Carbachol</td>
<td>15.8 ± 0.7</td>
<td>467 ± 38</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Atropine</td>
<td>15.1 ± 0.9</td>
<td>571 ± 52</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Atropine</td>
<td>15.4 ± 1.1</td>
<td>530 ± 56</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Carbachol</td>
<td>15.3 ± 1.0</td>
<td>502 ± 33</td>
<td>46 ± 8</td>
</tr>
</tbody>
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Results are expressed as mean ± SE.

Carbachol was infused at a rate of 1 µg/kg per min for 6 minutes; n = eight dogs.

Atropine (1 mg/kg) was administered 5 minutes before carbachol infusion; n = six dogs.

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Level of significance for changes in concentration of the metabolites at the end of infusion are as follows.

* P < 0.001
† P < 0.01
‡ P < 0.05
§ P < 0.02
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FIGURE 3 A recording from an electrically paced heart of a dog pretreated with atropine (1 mg/kg) followed in 5 min by intracoronal administration of carbachol (1 μg/kg per min). with no observable change in coronary blood flow or ventricular force. In addition to abolishing these hemodynamic effects, atropine blocked the carbachol-induced changes in uptake of blood substrates (Table 1). Neither atropine alone nor the subsequent infusion of carbachol significantly altered myocardial oxygen extraction ratio or uptake. Atropine treatment alone was found to cause a significant negative extraction of triglycerides, indicating that the heart was releasing the lipid substrate into its venous effluent. These data would suggest that, in addition to preventing changes in coronary blood flow and force of contraction, cholinergic receptor blockade in the heart is capable of influencing triglyceride metabolism.

An experiment representative of the hemodynamic responses recorded in one of the three reserpinized dogs that received a 6-min infusion of carbachol is shown in Figure 4. Hemodynamic changes associated with the infusion of carbachol were similar to those in Figure 1. However, there was a more pronounced increase in coronary flow in the three pretreated reserpinized dogs in response to carbachol than in the average coronary flow of the first group of dogs (93% vs. 46%). It was apparent that the vasodilating action was more pronounced in the absence of

the transmitter norepinephrine in peripheral and coronary blood vessels as shown by the more significant decrease in systemic and coronary perfusion pressure (Fig. 4). In the three reserpinized dogs infused with carbachol, the average myocardial uptake of oxygen slightly decreased from 4.2 to 3.7 ml/min per 100 g, while triglyceride uptake increased from —0.3 to 2.6 mg/min per 100 g and FFA uptake increased from 2.4 to 3.3 μEq/min per 100 g.

Figure 5 shows average changes in dogs on cardiopulmonary bypass in the percent incorporation of radioactive isotopes into myocardial intracellular FFA and triglycerides for five control dogs and six dogs receiving carbachol. At the end of the 6-min infusion of 14C-tripalmitin and 3H-FFA in dogs receiving a simultaneous infusion of carbachol, there was no significant increase in the amount of 3H-FFA that entered the myocardial pool of FFA. In addition, no change was evident in the rate of incorporation of 14C-tripalmitin into this FFA pool from the breakdown of triglycerides. However, the triglyceride pool into which 9.2 ± 1.1% of 3H-FFA was incorporated in the control group increased its rate of incorporating 3H-FFA 3-fold or by 27.6 ± 5.2% (P < 0.02) when carbachol was infused. As a result of the carbachol infusion, triglycerides that were taken up as 14C-tripalmitin also showed a significant increase in incorporation from a control level of 22.8 ± 4.8% to a level of 57.9 ± 10.4% (P < 0.02). Discussion

The intracoronary administration of carbachol to dogs subjected to cardiopulmonary bypass indicates that the resulting increase in coronary blood flow cannot be related to changes in coronary perfusion pressure, heart rate, or external cardiac work. The significant increase in coronary flow that was observed could be attributed mainly to stimulation of muscarinic receptors in coronary vessels. Stimulation of receptors in coronary vessels that were classified as muscarinic previously has been reported to result from the administration of ACh by intracoronary route in the open-chest dog.15 The reported experiments
show that the decrease in ventricular force of contraction caused by carbachol also can be attributed to stimulation of muscarinic receptors, since atropine was found to block this change.

In the absence of external cardiac work, dogs subjected to complete cardiopulmonary bypass were found to have a myocardial oxygen uptake of approximately one-third that of the working heart. The myocardial oxygen uptake of 4.2 ml/min per 100 g agrees with an estimation reported on the oxygen consumption for the beating empty dog heart. The decrease in force of contraction caused by carbachol was found to be accompanied by a 25% decrease in oxygen uptake. Both the decrease in force of contraction and oxygen uptake most likely reflect a carbachol-induced depression in utilization of myocardial metabolic substrates. While the metabolic details of cholinergic inhibition in the presence or absence of catecholamines remain to be explained, there are strong indications that the action of cholinergic agents modifies the metabolism of cardiac muscle in a way similar to what has already been reported for ACh. Acetylcholine is known to depress levels of myocardial cyclic AMP and at the same time elevate cyclic GMP. Whether increases in cyclic GMP by ACh can be responsible for depression of myocardial metabolism or whether the depression results from the lowered concentration of cyclic AMP remains unexplained. Glaviano et al. found that, in the dog heart, the intracoronary infusion of ACh in the presence of norepinephrine depressed the enzymic activity of adenylate cyclase and hormone-sensitive lipase. These enzymic changes in cardiac muscle were accompanied by a decrease in the myocardial uptake of oxygen and FFA.

Numerous investigators have found that the isolated perfused guinea pig heart, ACh inhibited the stimulatory action of epinephrine on glycogenolysis. Whether increases in cyclic GMP by ACh can be responsible for depression of myocardial metabolism or whether the depression results from the lowered concentration of cyclic AMP remains unexplained. Glaviano et al. found that, in the dog heart, the intracoronary infusion of ACh in the presence of norepinephrine depressed the enzymic activity of adenylate cyclase and hormone-sensitive lipase. These enzymic changes in cardiac muscle were accompanied by a decrease in the myocardial uptake of oxygen and FFA.
parameters are measured in the in situ heart. The implications that can be drawn from an altered state of neurohumoral control of cardiac metabolism would be that an imbalance in the heart favoring the parasympathetic components of the autonomic nervous system can lead to accumulation of myocardial triglycerides.

References
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