The antagonistic actions of the neurohumoral transmitters, norepinephrine and acetylcholine, on nodal, conducting and atrial tissue have been well established. Evidence for their direct antagonistic effects on ventricular contractility, however, has been more difficult to obtain. Using direct intracoronary infusions of sympathomimetic and parasympathomimetic drugs, we have obtained unequivocal evidence for such antagonism in the left ventricular myocardium. Acetylcholine blocked the positive inotropic effects of stellate ganglion stimulation and infused catecholamines. In addition to this blocking action, acetylcholine evoked a positive inotropic response which became apparent only when infusion of the drug was stopped.

Methods

Fasted mongrel dogs (25 to 45 kg) were anesthetized with sodium pentobarbital (25 to 30 mg/kg iv) and given additional doses as needed during the experiment. Artificial respiration was maintained by intermittent positive pressure through an endotracheal tube. The heart was exposed through a left thoracotomy, the pericardium opened, and the free margin sutured to the thoracic wall. Polyvinyl chloride catheters (O.D. 0.030 inch and I.D. 0.015 inch) were implanted in the left anterior descending (LAD) and left circumflex (LC) coronary arteries (fig. 1) by a modification of the method of Herd and Barger, with a small, side orifice in the intravascular segment of the catheter. The former was placed distal to the origin of the septal artery branch and the latter beyond the atrial branches to avoid arrhythmias and changes in heart rate. To record contractile force, two calibrated Walton-Brodie strain gauge arches were sutured to the left ventricular myocardium, one in the area supplied by the catheterized LAD artery (verified by intracoronary injection of indigo carmine) and the second in a region supplied by the LC artery. Femoral arterial blood pressure and heart rate were also recorded.

The following drugs, freshly dissolved in 0.9% saline, were infused into the LAD coronary artery at 0.25 to 0.5 ml/min using a constant infusion pump: norepinephrine bitartrate, acetylcholine chloride, physostigmine (salicylate or sulfate), atropine sulfate, hexamethonium bromide, bretylium tosylate, pronethalol, and bradykinin. When applicable, the dose is expressed as weight of the free base per minute. In some experi...
Intracoronary infusions of acetylcholine in low doses (0.5 to 20 \( \mu g/\text{min} \)) caused slight depression of myocardial contractility in some animals and no depression in others. When given, however, during the heightened contractions induced by intracoronary norepinephrine infusion, acetylcholine inhibited strikingly the positive inotropic effect of norepinephrine (fig. 3). In nine experiments on five dogs, infusion of norepinephrine (0.25 to 1.0 \( \mu g/\text{min} \)) into the LAD coronary artery increased the contractile force in the perfused area while contractility in the control area remained unchanged; heart rate and systemic arterial blood pressure were not altered. With the norepinephrine infusion continuing through one arm of the catheter, infusion of acetylcholine into the same coronary artery was started through the second arm. Within 20 to 30 seconds the augmented ventricular contractions in the perfused area began to decrease and reached control levels in two minutes. Similar results were obtained when epinephrine, tyramine, dopamine, or isoproterenol was used to increase ventricular con-
TABLE 1

Effect of Intracoronary Infusion (LAD) of Vasodilator Drugs on Myocardial Blood Flow

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>LAD area Control</th>
<th>During infusion into LAD</th>
<th>LC area Control</th>
<th>During infusion into LAD</th>
<th>Per cent increase of flow in LAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>0.025</td>
<td>92</td>
<td>130</td>
<td>82</td>
<td>71</td>
<td>41</td>
</tr>
<tr>
<td>(6 dogs)</td>
<td>0.50</td>
<td>89</td>
<td>104</td>
<td>73</td>
<td>63</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>92</td>
<td>132</td>
<td>82</td>
<td>90</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>69</td>
<td>154</td>
<td>73</td>
<td>66</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>69</td>
<td>189</td>
<td>—</td>
<td>—</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>87</td>
<td>213</td>
<td>78</td>
<td>66</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>75</td>
<td>177</td>
<td>66</td>
<td>60</td>
<td>136</td>
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<tr>
<td></td>
<td>25.0</td>
<td>154</td>
<td>378</td>
<td>—</td>
<td>—</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>52</td>
<td>238</td>
<td>120</td>
<td>130</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>80.0</td>
<td>154</td>
<td>378</td>
<td>120</td>
<td>122</td>
<td>145</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>0.25</td>
<td>154</td>
<td>222</td>
<td>120</td>
<td>121</td>
<td>44</td>
</tr>
<tr>
<td>(3 dogs)</td>
<td>0.50</td>
<td>154</td>
<td>245</td>
<td>154</td>
<td>154</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>154</td>
<td>208</td>
<td>77</td>
<td>73</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
<td>173</td>
<td>95</td>
<td>98</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>73</td>
<td>189</td>
<td>64</td>
<td>64</td>
<td>159</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>5.0</td>
<td>154</td>
<td>173</td>
<td>112</td>
<td>116</td>
<td>12</td>
</tr>
<tr>
<td>(2 dogs)</td>
<td>20.0</td>
<td>154</td>
<td>231</td>
<td>112</td>
<td>130</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>156</td>
<td>277</td>
<td>140</td>
<td>143</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>156</td>
<td>220</td>
<td>140</td>
<td>130</td>
<td>41</td>
</tr>
</tbody>
</table>

tractility. Since doses of acetylcholine that inhibited the positive inotropic effect of norepinephrine produced a 50 to 150% increase of myocardial blood flow (fig. 2B and table 1), the diminution of left ventricular contractile force produced by acetylcholine during norepinephrine infusion could have resulted from drug dilution rather than direct antagonism. Therefore, to maintain a constant arterial concentration of norepinephrine in the coronary artery during the increased blood flow produced by acetylcholine, norepinephrine was administered intravenously in a second set of experiments. In twenty experiments on ten dogs, norepinephrine (5 to 12.5 μg/min) was infused continuously into the femoral vein at a rate sufficient to stabilize blood pressure and force of left ventricular contraction at elevated levels (fig. 4). Acetylcholine (0.5 to 20 μg/min) was then infused directly into the LAD coronary artery. Within several seconds, contractility in the LAD area began to decrease while that in the control area remained the same, or increased slightly. Contractile force in the LAD area did not decrease to control values, probably the result of the elevated systemic blood pressure. Intracoronary acetylcholine (0.25 to 20 μg/min) also depressed the augmented contractile force produced by stimulation of the left stellate ganglion in ten experiments on four dogs (fig. 5). The antagonistic action of acetylcholine was blocked in three dogs by the intracoronary infusion of atropine sulfate (1 to 10 μg/min); the positive inotropic response to infused norepinephrine was not altered by atropine. Atropine also prevented the rise in myocardial blood flow ordinarily seen with intracoronary infusion of acetylcholine.

To provide evidence that the increase of myocardial blood flow produced by acetylcholine was not responsible for modifying the positive inotropic response to adrenergic stim-
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TIME IN MINUTES (LAD)

Effect of intracoronary (LAD) acetylcholine on the positive inotropic response to intravenous norepinephrine.

Two other potent vasodilators were used, bradykinin and sodium nitroprusside (table 1). Bradykinin (0.25 to 1.0 μg/min) infused into the coronary artery had no inotropic action in three dogs; it did not decrease the heightened force of ventricular contraction during intravenous infusion of norepinephrine or during stellate ganglion stimulation despite the increased coronary flow (fig. 5). Similar results (four experiments on two dogs) were obtained with intracoronary infusion of sodium nitroprusside (20 to 40 μg/min).

B. POSITIVE INOTROPIC ACTION OF ACETYLCHOLINE ON THE VENTRICULAR MYOCARDIUM

During control studies with intracoronary infusions of acetylcholine (0.5 to 20 μg/min) we noted that within seconds after the infusion was stopped the strength of ventricular contraction increased rapidly and strikingly (fig. 6). The positive inotropic effect ("rebound") reached a maximum in 12 to 20 seconds and then decreased during the next several minutes. Such responses were obtained repeatedly in 25 dogs, although the magnitude varied from animal to animal (in 4 dogs we failed to obtain any measurable positive inotropic response despite repeated placement of the strain gauge). Thus, intracoronary acetylcholine may release from the left ventricular myocardium a substance with positive inotropic action (? norepinephrine). The effects of the released substance are masked during the infusion of acetylcholine by the latter's antagonistic properties. The increased force of contraction became manifest when acetylcholine infusion was stopped, apparently because of the more rapid disappearance of acetylcholine than of the substance liberated. Evidence for such a thesis was provided by the intracoronary administration of physostigmine, an anticholinesterase drug which retards the degradation of acetylcholine. In six experiments on 4 dogs, physostigmine salicylate or sulfate (100 μg/min of the base) abolished or reduced strikingly the positive inotropic response ("rebound") when infusion of acetylcholine was stopped. Physostigmine alone did not change the force of ventricular contraction, nor did it alter the positive inotropic response to norepinephrine.

To help localize the site of action and possible structures involved in the acetylcholine "rebound," atropine, hexamethonium, bretylium tosylate, pronethalol, and reserpine were used. In 13 experiments on 5 dogs, intracoronary infusions of atropine sulfate (0.5 to 10 μg/min) completely blocked the "rebound" produced by acetylcholine (10 to 50 μg/min). The blockade persisted for approximately one hour after the infusion of atropine was stopped.

Hexamethonium bromide (0.5 to 1 mg/min, up to a total dose of 25 mg) infused into the coronary artery failed to block the "rebound" in eight experiments on four dogs; this dose was sufficient to decrease systemic pressure to 40 to 50 mm Hg. Bretylium administered into the LAD coronary artery of five dogs at a rate of 125 μg/min caused a slight, progressive increase in the force of ventricular contraction. After 15 to 20 minutes of...
Effects of intracoronary infusions of acetylcholine and bradykinin on left ventricular contractile force and myocardial blood flow (MBF) during left stellate ganglion stimulation.

Infusion, bretylium completely blocked the response to left stellate ganglion stimulation. At this time the usual positive inotropic response followed the cessation of acetylcholine infusion (fig. 7).

To determine whether the substance liberated from the ventricles by acetylcholine was adrenergic, pronethalol (a beta receptor blocking agent) and reserpine were employed. In ten experiments on six dogs, pronethalol was infused into the LAD coronary artery. Doses less than 1 mg/min produced no depression of contractility whereas greater amounts caused a 10 to 50% decrease in force of ventricular contraction. At a dose which caused little or no myocardial depression (0.5 to 1.0 mg/min), the drug effectively blocked the positive inotropic action of intracoronary norepinephrine (1.0 to 10 µg/min) and of tyramine (5 to 500 µg/min). In this dose range, however, pronethalol did not block the response to stellate stimulation or the "rebound" after acetylcholine (10 to 50 µg/min). A higher dose (2 mg/min) reduced the acetylcholine "rebound." However, the diminished "rebound" is difficult to interpret since the pronethalol had a significant negative inotropic effect at this rate of infusion.

To obtain additional information concerning the substance liberated by acetylcholine three reserpinized dogs were studied. One animal received reserpine (0.5 mg/kg im) 36 and 12 hours before the experiment while the other two had 0.1 mg/kg im 48 hours and 24 hours before the study. Acetylcholine still evoked a brisk "rebound" at the start of the experiment. Additional reserpine (intracoronary infusion of 50 µg/min for four and one-half hours) caused a progressive diminution and eventual abolition of the "rebound." At that time and dosage, however, contractility was markedly depressed, and intracoronary norepinephrine had little effect.
FIGURE 6
Effects of acetylcholine on ventricular contractility during, and immediately after, intracoronary infusion.

Discussion
Intracoronary infusion of acetylcholine has demonstrated a dual or biphasic action of the compound on ventricular muscle, a sympathomimetic effect as well as a sympathetic blocking action. While acetylcholine exerts only a minor, depressant effect on force of ventricular contraction during infusion, a marked positive inotropic response occurs immediately after the drug is stopped. Acetylcholine also antagonizes the positive inotropic response of the ventricular muscle to stellate stimulation or infusion of sympathomimetic agents such as epinephrine, norepinephrine, and tyramine. This antagonism represents a direct action on the ventricular myocardium, and does not depend on the known effects of acetylcholine on the atria and vascular smooth muscle, nor on its coronary vasodilator action. Bradykinin and sodium nitroprusside, which produced similar increases in coronary blood flow, did not modify the positive inotropic response to catecholamines.

These findings of acetylcholine-norepinephrine interaction and antagonism complement recent studies on the biochemical effects of such neurohormonal interaction on the heart. Murad et al., using subcellular, particulate preparations of dog and guinea pig hearts, showed that acetylcholine inhibited the formation of cyclic 3', 5' adenosine monophosphate. This inhibition occurred in the presence of epinephrine in amounts usually sufficient to stimulate the brisk formation of this substance. Although the role of cyclic 3', 5' adenosine monophosphate in cardiac contractility is presently unclear, the demonstration of such biochemical antagonism between acetylcholine and epinephrine at the cellular and subcellular levels is of great interest. Vincent and Ellis, employing perfused guinea pig hearts whose ventricles were driven electrically at a constant rate, showed that acetylcholine, when given alone, did not change cardiac glycogen concentration. When given together with epinephrine, however, acetylcholine strikingly antagonized the glyco genolytic action of epinephrine. Such findings are analogous to those of the present study.

The second action of acetylcholine on ventricular muscle, i.e., its ability to increase the force of ventricular contractions, is probably a result of liberation from ventricular myocardium of a substance with positive inotropic action. The inotropic action is blocked during infusion of acetylcholine by the antagonistic properties of this compound as described above. More forceful contractions became manifest only after the cessation of acetylcholine infusion, and are due probably to more rapid disappearance of acetylcholine than of the unknown substance liberated. Physostigmine abolishes the "rebound" by retarding the inactivation of acetylcholine and extending its antagonistic actions. Alternatively, physostigmine may prevent the "rebound" by a mechanism independent of its anticholinesterase properties.

Investigators have noted the ability of acetylcholine or vagal stimulation to augment car-
Effect of intracoronary infusion of bretylium on responses to left stellate ganglion stimulation and intracoronary acetylcholine. After bretylium completely blocked the response to stellate stimulation in the perfused area, acetylcholine still elicited a vigorous "rebound."

diac contractility, especially in the atropinized animal.11-14 Hoffman et al.,11 using dog heart-lung preparations and cat, rabbit, and guinea pig Langendorff preparations, showed in the atropinized heart that acetylcholine induced more forceful contractions, increased the heart rate, and liberated an epinephrine-like substance. The active substances recovered in the perfusate, like epinephrine, caused an increase in the force of contraction of the hypodynamic frog heart, and relaxation of both the rectal cecum of the fowl and the small intestine of the rabbit. Middleton et al.,13 using atropinized, isolated cat hearts, obtained similar results with either vagal stimulation, intracoronary injection of acetylcholine, or pre- and postganglionic cardiac sympathetic stimulation. Because nicotine abolished its cardio-stimulating effects, they concluded that vagal stimulation released the epinephrine-like substance by exciting adrenergic intracardiac ganglion cells. In a later study,14 however, they observed no ganglion cells or chromaffin tissue in serial sections from cat papillary muscles which had exhibited a positive inotropic response to acetylcholine. Lee and Shideman15 also failed to find ganglion cells in serial sections of cat papillary muscles which had responded to acetylcholine with increased force of contraction. Our present findings also suggest that acetylcholine acts by exerting its positive inotropic action not on ganglia but at a peripheral site. Atropine, which blocks the muscarinic actions of acetylcholine, inhibited the effects of acetylcholine (i.e., the antagonism to norepinephrine, the augmentation of ventricular contractility, and the increase of myocardial blood flow). Large intracoronary doses of hexamethonium, which produced significant hypotension, did not block the "rebound" after acetylcholine infusion. Bretylium, in doses that completely blocked the response
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to stellate ganglion stimulation, also failed to block the acetylcholine induced augmented cardiac contractions. Bretylium blocks the response to sympathetic stimulation by acting on the terminal portion of the sympathetic neurone and by preventing the release of norepinephrine. Thus, if the substance liberated is indeed norepinephrine, acetylcholine must exert its action distal to the site of bretylium block, perhaps on the norepinephrine binding sites themselves. The mode and site of action of acetylcholine thus resemble that of tyramine, which also releases norepinephrine despite adrenergic blockade due to bretylium as shown by Gilmore and Siegel. Although tyramine and acetylcholine appear to act at a site distal to the action of bretylium, they are dissimilar in effect since the positive inotropic response to tyramine is easily blocked by pronethalol while that due to acetylcholine is not affected.

We attempted to establish the catecholamine nature of the substance released by using an adrenergic beta receptor blocking agent, pronethalol. Although it blocked effectively the positive inotropic response to various sympathomimetic drugs infused directly into the coronary arteries, it failed to block responses of equivalent magnitude evoked by left stellate ganglion stimulation or by the cessation of intracoronary acetylcholine infusion. Our inability to block the response to stellate stimulation with pronethalol in doses which did not exert severe cardiodepressor effects contrasts with the efficacy of such blockade originally reported by Black and Stephenson. Thus, pronethalol blocks the effects of sympathetic nerve stimulation less effectively than those due to circulating catecholamines. Ferry has shown in the spleen that acetylcholine excites sympathetic postganglionic nerve fibers near their endings, causing not only a sympathetic response with liberation of norepinephrine but also antidromic propagation of impulses. Similarly, acetylcholine, by depolarizing the terminal C-fiber endings in ventricular myocardium, might initiate an axon reflex which would spread beyond the drug perfused area. This would then release the unknown substance from more remote areas not accessible to the actions of infused pronethalol. Alternatively, we cannot rule out the possibility that the substance liberated is structurally or functionally unlike other sympathomimetic compounds. Moreover, in animals given reserpine in doses known to deplete the heart of 98% of its endogenous catecholamines, the acetylcholine induced "rebound" was still obtainable. This, too, suggests a noncatecholamine nature of the unknown substance. However, the small amount of norepinephrine remaining, if strategically located, may be sufficient to evoke the postacetylcholine augmented contractions. In addition, we have recently observed (unpublished observations) that direct intracoronary infusion of acetylcholine into the chronically catheterized coronary artery of dogs produces electrocardiographic changes identical to those seen during intracoronary infusion of epinephrine and norepinephrine. In agreement with the present studies, pretreatment with reserpine for 24 to 48 hours did not prevent the electrocardiographic changes induced with acetylcholine.*

Many workers have demonstrated sympathomimetic effects of acetylcholine in organs other than the heart. Burn and Rand suggested that such action was of general physiologic importance and that acetylcholine played an intermediate role in postganglionic sympathetic nerve transmission. They postulated that postganglionic nerve impulses release acetylcholine which, in turn, releases norepinephrine in the vicinity of nerve endings. Folkow et al. showed that during stimulation of the stellate ganglion, a substance with acetylcholine-like properties appeared in the coronary perfusate of the dog and cat. He therefore postulated the presence of cholinergic fibers in the sympathetic outflow to the

*In two experiments the total catecholamines in the coronary venous blood draining the perfused area were determined by Dr. Richard Croft using his modification of the trihydroxyindole method. However, during and after acetylcholine infusion the catecholamine levels were too low to determine significant differences from control levels.
ACETYLCHOLINE ON VENTRICULAR MYOCARDIUM

heart. In this context our findings may suggest that cholinergic fibers within the sympathetic nerves could ultimately exert adrenergic effects.

The demonstrated cardio-inhibitory and cardio-excitatory actions of acetylcholine in dog ventricle provide another possible mechanism for regulation of cardiac function. Stimulation of either sympathetic cholinergic or adrenergic fibers might release varying quantities of both acetylcholine and norepinephrine. The magnitude and direction of inotropic response of the end organ would depend on the relative amounts of each neurohumor released, and on their interaction.

Summary

Intracoronary infusions of acetylcholine have demonstrated two actions of this neurohumoral agent on ventricular myocardium: 1) an antagonism to the more forceful contractions evoked by adrenergic stimuli (sympathomimetic drugs or stellate stimulation), and 2) a sympathomimetic or positive inotropic effect (? release of a positive inotropic substance from the myocardium). The increased contractility ("rebound") was observed only after the infusion of acetylcholine was stopped; in the presence of physostigmine the "rebound" was absent. Atropine blocked both the cardio-inhibitory and cardio-excitatory actions of acetylcholine. Failure of hexamethonium and bretylium to block the "rebound" suggests that acetylcholine acts at a peripheral site. The possible catecholamine nature of the agent could not be established by direct analysis of coronary venous blood, by the use of an adrenergic beta receptor blocking agent or by pretreatment of the animals with reserpine; neither drug blocked the "rebound."

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