Effects of Intracoronary Acetylcholine and Atropine on Basal and Dobutamine-Stimulated Left Ventricular Contractility

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Background  The role of cholinergic pathways in modulating left ventricular contractile function in humans is not known. This study evaluated the effect of a cholinergic agonist (acetylcholine) and antagonist (atropine) on basal and \( \beta \)-adrenergically stimulated left ventricular contractile function in normal subjects and subjects with denervated hearts after cardiac transplantation.

Methods and Results  Six subjects with normal left ventricular function and seven subjects who were 1 to 3 years after cardiac transplantation were studied. Acetylcholine, atropine, and the \( \beta \)-adrenergic agonist dobutamine were infused via the left main coronary artery, and changes in left ventricular contractile function were assessed by measurement of peak +dP/dt. Intracoronary dobutamine increased +dP/dt by 70±15% and 66±20% in the normal subjects and transplant recipients, respectively. Intracoronary acetylcholine and atropine alone each had no effect on left ventricular +dP/dt in either normal subjects or transplant recipients. The concurrent infusion of acetylcholine with dobutamine reduced the response to dobutamine by 66±10% and 79±9% in normal subjects and transplant recipients, respectively. The concurrent infusion of atropine with dobutamine potentiated the response to dobutamine by 25±7% in normal subjects but had no effect in transplant recipients.

Conclusions  Stimulation and inhibition of cholinergic receptors in the human heart can modulate the positive inotropic response to \( \beta \)-adrenergic stimulation. (Circulation. 1994;89:164-168.)

Key Words:  * left ventricle  * acetylcholine  * dobutamine

tential action of acetylcholine or atropine to affect cardiac function by modulating presynaptic norepinephrine release, seven subjects who had undergone cardiac transplantation from 1 to 3 years previously.

Methods

Study Population  The study population consisted of 13 adults (10 men and 3 women) who were referred for diagnostic catheterization. All subjects were in normal sinus rhythm. Six of the subjects (normal subjects) were referred for evaluation of a chest pain syndrome. In all of these subjects, the coronary arteries were found to be free of significant stenosis by angiography. Five of these subjects were free of symptoms of left ventricular dysfunction or congestive heart failure and had received medications only for treatment of chest pain (diltiazem, one subject; verapamil, one subject; and atenolol, one subject). One subject had chronic renal insufficiency and had received digoxin and captopril for pulmonary congestion but was found to have normal left and right ventricular size and function by echocardiography and had normal resting hemodynamics at the time of study. All medications were withheld on the night before study, except atenolol, which was withheld for 48 hours before study. Left ventricular ejection fraction determined by angiography (three subjects), echocardiography (two subjects), or radionuclide ventriculography (one subject) was normal in all six subjects. A second group of seven subjects (transplant recipients) were studied during their routine annual evaluation after cardiac transplantation. These subjects were studied from 1 to 3 years after transplantation (1 year, four subjects; 2 years, two subjects; and 3 years, one subject) and in all cases were free of clinical or histological evidence of cardiac rejection. The coronary arteries in these subjects were without significant stenosis by angiography. The medications in this group consisted of prednisone and cyclosporine A in all subjects. In addition, several subjects were receiving a convert-
ing enzyme inhibitor (three subjects), a calcium channel antagonist (four subjects), or both (two subjects) for the control of high blood pressure. All medications were withheld on the night before study.

The study protocol was approved by the Committee for the Protection of Human Subjects From Research Risks at the Brigham and Women’s Hospital, and written informed consent was obtained before study.

**Hemodynamic Measurements**

All subjects initially underwent routine diagnostic left and right heart catheterization via the femoral approach. Coronary angiography was performed with nonionic contrast media, and the research protocol was begun a minimum of 20 minutes after completion of the diagnostic catheterization. The methodology for intracoronary drug infusion and hemodynamic measurements was as reported previously. In brief, a 6F L-4 Judkins catheter (Cordis Laboratories, Miami, Fla) was advanced to the ostium of the left main coronary artery and positioned as for routine contrast injection. The catheter was continuously flushed with 5% dextrose in water (D5W) infused at a rate of 2 mL/min. A 7F micromanometer-tipped pigtail catheter (Millar Industries, Houston, Tex) was advanced from the opposite femoral artery and placed in the left ventricle for measurement of intracavitary left ventricular pressure. Left ventricular peak +dP/dt was computed on-line by an Electronics for Medicine model 220A amplifier (Honeywell, Inc, Pleasant Valley, NY). Femoral arterial pressure was monitored via an 8F side-arm sheath (Cordis Laboratories).

The ECG, femoral arterial pressure, left ventricular pressure, and peak +dP/dt were recorded on a strip-chart recorder (Electronics for Medicine, Honeywell, Inc, Pleasant Valley, NY). Each measurement is the mean of at least 10 consecutive sinus beats under each experimental condition, except for +dP/dt, which was the average of least 45 consecutive beats.

**Drug Infusion Protocols**

Drugs were infused into the left main coronary artery via the Judkins catheter using a Harvard pump (South Natick, Mass) as previously described. Each drug was infused for 4 to 5 minutes with hemodynamic measurements made during the last minute of drug infusion. The sequence of drug infusions was as follows: (1) D5W, the vehicle for intracoronary drugs, was infused at a rate of 2 mL/min. (2) Dobutamine (Lilly, Inc, Indianapolis, Ind) diluted in D5W was infused at a rate of 12.5 or 25 μg/min, as characterized in detail previously, so as to increase +dP/dt by at least 50%. The infusion rate was 12.5 μg/min in all except two normal subjects and one transplant recipient who received 25 μg/min. (3) Dobutamine was discontinued and +dP/dt was monitored for at least 5 minutes and until return to the initial baseline value. (4) Acetylcholine was infused at 28.3 μg/min, a rate calculated to yield a steady-state coronary artery concentration of 1 μmol/L based on an assumed left main coronary artery blood flow of 125 mL/min. (5) After the hemodynamic measurements had been made during acetylcholine infusion, acetylcholine was continued with the concurrent infusion of dobutamine at the rate used for the first dobutamine infusion. (6) Acetylcholine and dobutamine were discontinued for a minimum of 5 minutes and until +dP/dt had returned to baseline. (7) Atropine was infused at a rate of 12 μg/min. (8) After hemodynamic measurements had been made during atropine infusion, atropine was continued with the concurrent infusion of dobutamine at the rate used for the first dobutamine infusion. (9) After completion of the drug infusions, radiographic contrast was injected to confirm the continued position of the catheter in the left main coronary ostium.

**Statistical Analysis**

All data are presented as the mean±1 SEM. The significance of changes in each variable was tested by ANOVA for multiple observations. Intragroup and intergroup comparisons were made by paired or unpaired two-tailed Student’s t tests, as appropriate. The statistical significance of differences in multiple observations was tested by means of the Bonferroni correction for repeated measures, such that a P value of <.05/n was required for statistical significance, where n is the number of comparisons tested.

**Results**

**Baseline Hemodynamics**

Baseline hemodynamics in the six normal subjects were within normal limits (Table 1). In the seven transplant recipients, baseline hemodynamics were within normal limits, with the exception of left and right heart filling pressures, which were mildly elevated. There were no significant differences between normal subjects and transplant recipients, with the exception of mean pulmonary artery wedge pressure, which was higher in the transplant recipients.

**Effects of Intracoronary Dobutamine, Acetylcholine, and Atropine on Basal Contractility**

Heart rate, left ventricular end-diastolic pressure, and mean arterial pressure were unaffected by any of the intracoronary drug infusions in normal subjects or transplant recipients (Table 2). Dobutamine infusion increased left ventricular +dP/dt by 70±15% and 66±20% in the normal subjects and transplant recipients, respectively (Fig 1). Discontinuation of dobutamine resulted in a return of +dP/dt to control values.
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In normal subjects, the infusion of acetylcholine (Ach, 28.3 μg/min), atropine (Atr, 12 μg/min), or combinations thereof, as indicated. Control infusions (Con) consisted of 5% dextrose in water. Changes in +dP/dt were significant by ANOVA in both groups.

Discussion

These data demonstrate that cholinergic agonists and antagonists can modulate the effects of β-adrenergic stimulation on left ventricular contractility in humans. The positive inotropic response to β-adrenergic receptor stimulation with dobutamine was markedly attenuated by the infusion of acetylcholine and significantly augmented by atropine. In contrast, under basal conditions (eg, in the absence of exogenous β-adrenergic receptor stimulation), neither acetylcholine nor atropine had a detectable effect on left ventricular contractility. Thus, the positive inotropic response to β-adrenergic receptor stimulation is significantly modulated by stimulation and inhibition of cholinergic receptors in the heart.

Work by several investigators has demonstrated a significant sympathetic-parasympathetic interaction in the regulation of left ventricular function in dogs. Levy and colleagues1,2,4,5 showed that vagal nerve stimulation inhibited the positive inotropic response to sympathetic nerve stimulation, that the inhibitory effect of parasympathetic nerve stimulation was greater than would be predicted by pure additivity, and that the effect of vagal stimulation was increased as the level of sympathetic activity was raised, a phenomenon they called "accentuated antagonism." Hollenberg et al3 showed that intracoronary infusion of acetylcholine in anesthetized dogs had little effect on basal left ventricular +dP/dt but significantly attenuated the positive inotropic responses to both sympathetic nerve stimulation and exogenously administered norepinephrine. Vatner et al6,7 extended

![Graph](http://circ.ahajournals.org/)

**Fig 1.** Plot of left ventricular peak +dP/dt in six normal subjects (NL) and seven transplant recipients (TX) in response to intracoronary infusions of dobutamine (Dob, 12.5 to 25 μg/min), acetylcholine (Ach, 28.3 μg/min), atropine (Atr, 12 μg/min), or combinations thereof, as indicated. Control infusions (Con) consisted of 5% dextrose in water. Changes in +dP/dt were significant by ANOVA in both groups.

**Fig 2.** Bar graph of effect of intracoronary acetylcholine (ACH) on the positive inotropic response to intracoronary dobutamine (DOB) in normal subjects and transplant recipients. The increase in +dP/dt with intracoronary DOB was significantly reduced by concurrent infusion of ACH with DOB in both normal subjects and transplant recipients (*P < .025 vs DOB alone).
these observations to conscious dogs by showing that atropine significantly potentiated the positive inotropic responses to several β-adrenergic agonists and that this effect did not require an intact baroreflex pathway. Our findings are consistent with these prior observations in dogs and, for the first time in humans, demonstrate the importance of cholinergic pathways in modulating left ventricular contractile function.

There are several potential mechanisms that may account for the effect of acetylcholine we observed. First, stimulation of cholinergic receptors on presynaptic sympathetic nerve endings may cause a decrease in the release of norepinephrine in response to nerve stimulation. Second, stimulation of muscarinic cholinergic receptors on the cardiac myocyte may cause a decrease in adenylate cyclase activity. Third, cholinergic receptors on endothelial cells and/or other cardiac cell types may cause the release of one or more substances (eg, endothelium-derived relaxing factor) that may interact with cardiac myocytes. In this regard, Balligand et al have shown in cardiac myocytes that acetylcholine inhibits the heart rate and contractile responses to a β-adrenergic agonist by a mechanism that involves nitric oxide synthesis. A role for nitric oxide in mediating autonomic responsiveness of the heart is also supported by the observation of Hare et al that in dogs, the positive inotropic response to intracoronary isoproterenol is potentiated by concurrent infusion of Nω-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase.

The effects of acetylcholine reported here are probably not due to a presynaptic site of action since acetylcholine was equally effective at decreasing the positive inotropic responses to dobutamine in transplanted and normal hearts. To the extent that the transplanted heart is denervated, this observation suggests that a presynaptic mechanism was not involved. Although there is evidence of partial sympathetic reinnervation in some patients after transplantation, analysis of the heart rate variability of donor hearts has not shown evidence of vagal reinnervation. Furthermore, in our transplant recipients, atropine failed to potentiate the response to dobutamine, as it did in normal subjects. Because our experiments were conducted in subjects under resting conditions, we cannot exclude the possibility that a presynaptic interaction might occur in other situations associated with increased sympathetic nerve activity (eg, exercise or heart failure). Based on the effects of sympathetic and parasympathetic nerve stimulation in dogs, it is likely that if acetylcholine exerts a presynaptic effect in humans, it would be most evident with increased sympathetic nerve activity.

Certain experimental limitations need to be considered. First, left ventricular peak +dP/dt, the measure of contractility used in these studies, can be influenced by changes in heart rate and loading conditions. This potential confounding factor appears to have had little effect since heart rate, systemic arterial pressure, and left ventricular end-diastolic pressure were unaffected by any of the intracoronary infusions under the conditions of these studies. A second limitation is that both the d- and l-stereoisomers of racemic (dl) dobutamine, the β-adrenergic agonist used in these studies, can also interact with α-adrenergic receptors. Since α-adrenergic receptor stimulation exerts a positive inotropic effect in human hearts, this could potentially confound the interpretation of the pharmacology demonstrated here. However, this possibility is unlikely since we have found that the positive inotropic effect of intracoronary dl-dobutamine is not affected by the α-adrenergic antagonist phentolamine, likely reflecting the opposing actions of the d- and l-isomers of dobutamine on α-adrenergic receptors. Finally, the endothelium-dependent vasodilatory effect of acetylcholine may have contributed to the decreased inotropic response to dobutamine by causing a reduction in the final intracoronary concentration of the drug. However, we think it is unlikely that this mechanism played a major role because (1) the magnitude of change in coronary blood flow with acetylcholine is not sufficient to account for the magnitude of decrease in dobutamine response that was observed, and (2) a comparable or greater effect of acetylcholine was seen in transplant recipients and patients with dilated cardiomyopathy (unpublished data) who have a reduced endothelium-dependent vasodilatory response to acetylcholine.

In summary, we have shown that cholinergic receptors in the human heart can modulate the positive inotropic response of the left ventricle to β-adrenergic receptor stimulation. These observations have implications with regard to the assessment of left ventricular physiology and pharmacology and provide a mechanism by which exogenous cholinergic agonists or antagonists or alterations in endogenous parasympathetic tone might influence left ventricular function.

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