*Br. J. Pharmac.* (1970), **38**, 93–105.

# Cholinergic mechanisms on the heart and coronary circulation

MARTA I. BLESA AND G. ROSS

*Department of Physiology, U.C.L.A. School of Medicine, Los Angeles, California 90024*

## Summary

1. The effects of rapid intracoronary injection of acetylcholine (ACh) were studied in anaesthetized open chest dogs. Changes in phasic coronary blood flow were followed with non-cannulating electromagnetic flow probes and in contractile force with isometric strain gauges.

2. Increasing doses of ACh from 0·01 to 100 $\mu$g produced progressively larger increases in systolic and diastolic coronary blood flow and progressive decreases in end-diastolic vascular resistance which were blocked by atropine but not by propranolol.

3. Contractile force showed both negative and positive responses. The negative inotropic effect was small and was blocked by atropine but not by propranolol. The threshold for the negative inotropic response was higher than for the coronary vasodilator effect and the dose response curve was flatter. The positive inotropic response usually showed two components. One component reached its maximum 13 to 18 s after injection, had a high threshold (over 1 $\mu$g), was potentiated by atropine and blocked by propranolol. The other reached its maximum 25 to 60 s after the injection, had a threshold between 0·01 and 0·1 $\mu$g, and was blocked by atropine but not by propranolol.

4. These results suggest that the coronary dilator response, the negative inotropic response and part of the positive inotropic response are mediated through " muscarinic " receptors. The remaining component of the positive inotropic response appears to involve catecholamine release.

## Introduction

Although the effects of acetylcholine (ACh) on the heart have been extensively studied, its mechanism of action remains unclear. Thus, Buccino, Sonnenblick, Cooper & Braunwald (1966) concluded from studies on isolated rat atria and papillary muscles that two distinct cholinergic receptors were present in the myocardium. Type I receptor responded to small doses of ACh and gave a negative inotropic effect which was blocked by atropine. Type II responded to larger doses, exerted a positive inotropic effect and was not blocked by atropine. On the other hand, Blumenthal, Wang, Markee & Wang (1968) have reported that the ventricular responses of the intact heart to ACh did not substantiate the existence of two types of cholinergic receptor. Instead they concluded that the negative inotropic response was a direct effect of ACh, whereas the positive inotropic response was due to catecholamine release. This view has also been supported by studies with dogs in

sinus rhythm and in heart block (Eliakim, Bellet, Tawil & Muller, 1961) and with cat papillary muscles (Middleton, Oberti, Prager & Middleton, 1956).

Because of these apparently conflicting results, we decided to assess the effects of ACh on ventricular contractile force and coronary blood flow using a wide range of doses in a relatively intact preparation. The ACh was injected directly into the anterior descending coronary artery so as to minimize or eliminate heart rate and arterial pressure changes. Atropine and propranolol were used in an attempt to clarify the nature of the observed responses.

## Methods

Twenty dogs weighing 10–18 kg were anaesthetized with pentobarbital sodium 40 mg/kg intravenously and intubated. The chest was opened in the fifth left intercostal space, while ventilation was maintained with a Bird Mark 8 respirator. After incising the pericardium, the anterior descending branch of the left coronary artery was carefully dissected over a distance of approximately 3 cm and a non-cannulating electromagnetic flow probe was placed on the vessel. Flow was determined by means of a Biotronex BL-610 flowmeter. The zero reference point was frequently determined during each experiment by mechanically occluding the vessel distal to its probe. Calibration was performed by placing the probe on a branch of a femoral artery before killing the animal and withdrawing blood from this vessel at a constant rate with a Harvard infusion-withdrawal pump.

Aortic pressure was determined with a Statham P23Db transducer via a polyethylene tube inserted into the carotid artery and advanced into the aorta. The frequency response of both the flow and pressure measuring systems was 25 Hz ($\pm 3$ db). Acetylcholine was injected directly into the coronary artery through an indwelling 30 gauge needle in doses of 0·01, 0·1, 1·0, 10 and 100 $\mu$g. The needle had no effect on either mean or instantaneous coronary flow. Supplementary anaesthetic and blocking agents were injected through a catheter in a jugular vein.

A Walton-Brodie strain-gauge was sutured on a portion of myocardium within the area of distribution of the anterior descending coronary artery so that changes in contractile force produced by acetylcholine could be determined at the same time as the coronary flow changes. All variables were registered on a Beckman SII Dynograph recorder.

Diastolic and systolic coronary blood flows were calculated by measuring the appropriate area under the flow curve. Diastole was considered to start with the inflection in the flow trace coincident with the closure of the aortic valves and marked by the incisura of the aortic pressure record. Systole was considered to begin with the onset of coronary flow deceleration immediately preceding the systolic upstroke of the pressure curve. All measurements were averaged over three cycles. End diastolic vascular resistance (EDVR) was computed from records taken at fast paper speed by dividing the coronary flow at the end of diastole into the simultaneous value of aortic pressure.

The drugs used were: acetylcholine chloride (Merck & Co., Inc.); propranolol hydrochloride (Ayerst Laboratories, Inc.); atropine sulphate and isoprenaline hydrochloride (Isuprel, Winthrop-Stearns, Inc.). Doses are expressed in terms of the salts.

**Results**

The mean control value and the S.E. for pressure, heart rate and EDVR for the twenty dogs were:

Systolic pressure = 127 ± 4 mm Hg

Diastolic pressure = 98 ± 3 mm Hg

Heart rate = 150 ± 7 beats/min

EDVR = 3·68 ± 0·30 mm Hg/ml. per min

*Effects of ACh on coronary blood flow*

The threshold dose for eliciting a coronary blood flow response varied between 0·005 and 0·01 μg. All doses above threshold increased mean coronary blood flow over the entire duration of the response, which began within 2–4 s of injection, reached a peak within 4–8 s and returned to normal within 100–200 s, depending on the dose. Diastolic coronary flow was increased and end diastolic vascular resistance was decreased throughout. Systolic coronary flow was initially increased but with doses of ACh above 10 μg systolic flow frequently diminished in association with a reduction in arterial pressure, an increase in myocardial contractile force or both. The typical changes in instantaneous coronary flow following a large dose are shown in Fig. 1 and the changes in end-diastolic vascular resistance induced by a wide range of doses is shown in Fig. 2.

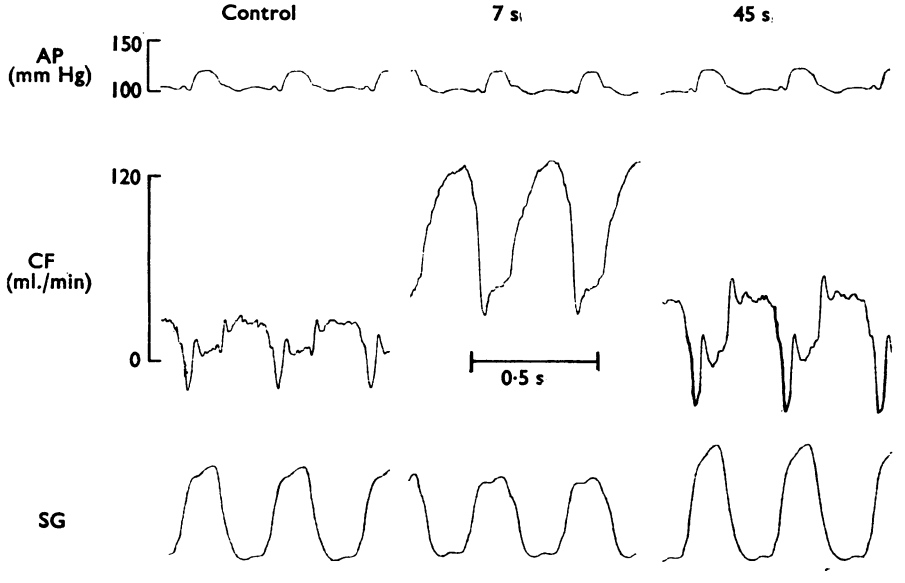


FIG. 1. Effect of intracoronary ACh 10 μg on instantaneous systemic arterial pressure (AP), anterior descending coronary arterial flow (CF), and left ventricular contractile force (SG). Segments of recordings taken before and 7 and 45 s after injection are shown. At 7 s both diastolic and systolic coronary flow have increased and contractile force is reduced. At 45 s there is an increase in contractile force and diastolic flow, but systolic flow is now below control and systolic back flow has increased. Aortic pressure and heart rate were unchanged throughout the response in this experiment.

*Contractile force changes*

The contractile force changes were complex and for a given dose there was much variation among the animals (Fig. 3). The character of the response in a single animal also varied with dose (Fig. 4). Initially, a small and brief negative inotropic effect beginning 2–4 s after the onset of the coronary flow change was observed with increasing frequency as the dose was raised from 0·01 to 10 $\mu$g. In contrast with

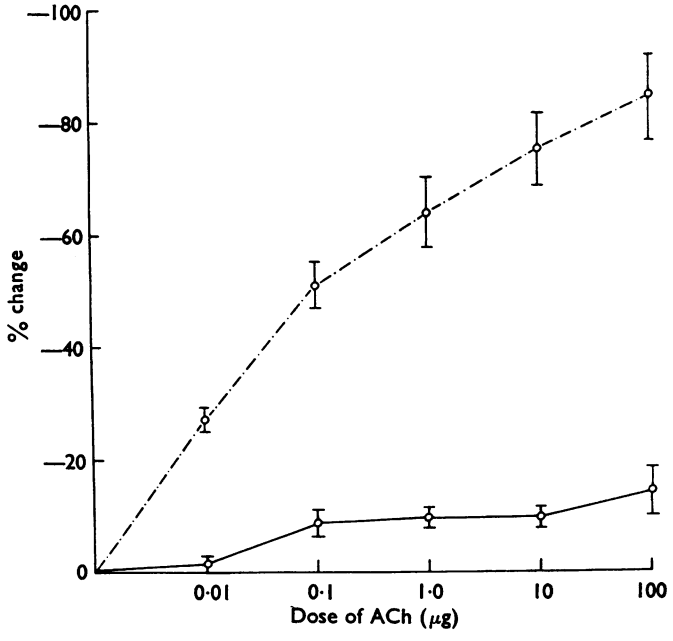


FIG. 2. Dose-response curve for end diastolic coronary resistance (EDVR) (○—·—○) and the negative inotropic response (○——○). Both responses are expressed as % change from control. Means and standard errors are shown.

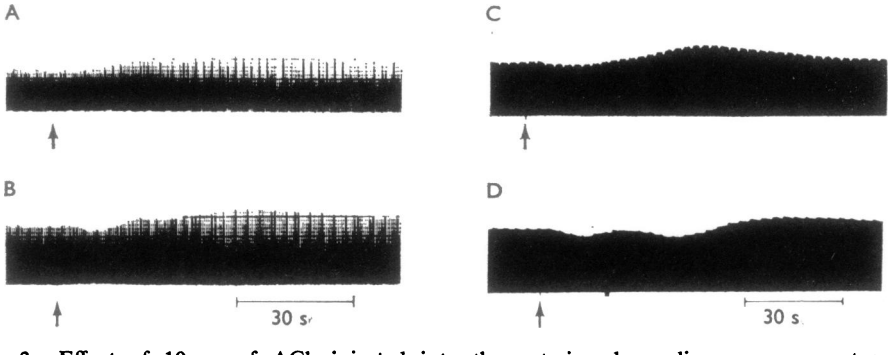


FIG. 3. Effect of 10 $\mu$g of ACh injected into the anterior descending coronary artery on ventricular contractile force in each of four different dogs. Note the variability: A, first and second inotropic peaks, no negative inotropic effect; B, negative followed by two positive inotropic peaks; C, slight negative followed by a single positive inotropic peak; D, prolonged negative inotropic response interrupted by the first " positive " inotropic peak and followed by the second inotropic peak.

the coronary flow change the dose response curve of this negative inotropic response was very flat (Fig. 2). A pure negative inotropic effect (one not followed by a positive inotropic response) was observed in only one of twenty animals. On the other hand, a positive inotropic response could be elicited in all animals by an appropriate dose. It began 2–12 s after the onset of the coronary flow change and

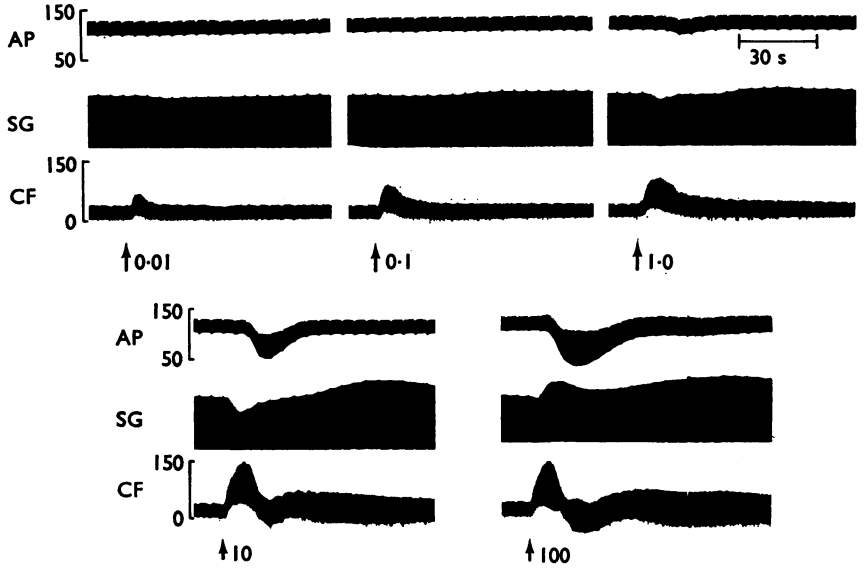


FIG. 4. Effect of increasing doses (μg) of ACh on the same dog, on aortic pressure (AP), coronary flow (CF) and contractile force (SG). Only negative and late positive inotropic responses are present with 1 μg or less, with 10 and 100 μg an early positive response is also present. All doses increase coronary blood flow but 10 and 100 μg produce systolic back flow during part of the response.

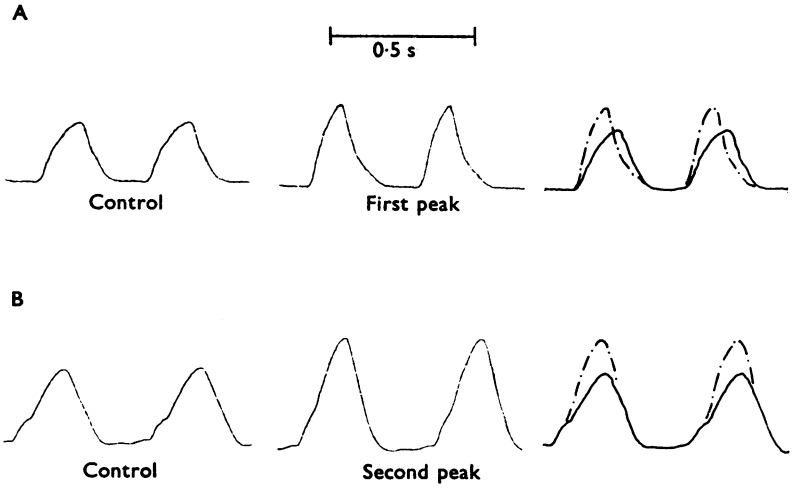


FIG. 5. Contractile force changes during first (A) and second (B) inotropic responses to intracoronary ACh 10 μg in different dogs. To illustrate the differences more clearly the instantaneous tension records during the response are superimposed on the pre-injection records. During the first inotropic peak, time to maximum tension is shortened from 145 to 100 ms. During the second inotropic peak, time to maximum tension changes from 170 to 160 ms.

often showed two " humps " (Fig. 3A, B and D). The first reached a maximum, subsequently referred to as the " first peak ", 13–18 s after injection, and the threshold dose for this effect lay between 1 and 10 $\mu$g. Examination of records at fast paper speed showed that the time to peak tension was reduced at this time (Fig. 5). The second " hump " reached a maximum 25 to 65 s after the injection and will be referred to as the " second peak ". Its threshold lay between 0·01–0·1 $\mu$g. Time to peak tension at this time did not differ significantly from the pre-injection value (Fig. 5). When the positive inotropic response showed only one " hump " the maximum was invariably found to occur later than 25 s after the injection; that is, it corresponded to the " second peak ". The incidence of the various types of myocardial response is shown in Table 1. It will be seen that all

TABLE 1. *Type of inotropic response to ACh*

| Dose ($\mu$g) | No. of dogs | Negative only | Negative + single positive, maximal at 35–65 sec | Negative + two positive peaks | Positive only, maximal at 30–65 sec | Two positive peaks only | No response |
|---|---|---|---|---|---|---|---|
| 0·01 | 19 | 2 | 1 | 0 | 0 | 0 | 15 |
| 0·1 | 18 | 1 | 6 | 0 | 5 | 0 | 6 |
| 1·0 | 19 | 0 | 12 | 1 | 2 | 4 | 0 |
| 10·0 | 20 | 0 | 10 | 6 | 0 | 4 | 0 |
| 100·0 | 16 | 0 | 4 | 3 | 0 | 9 | 0 |

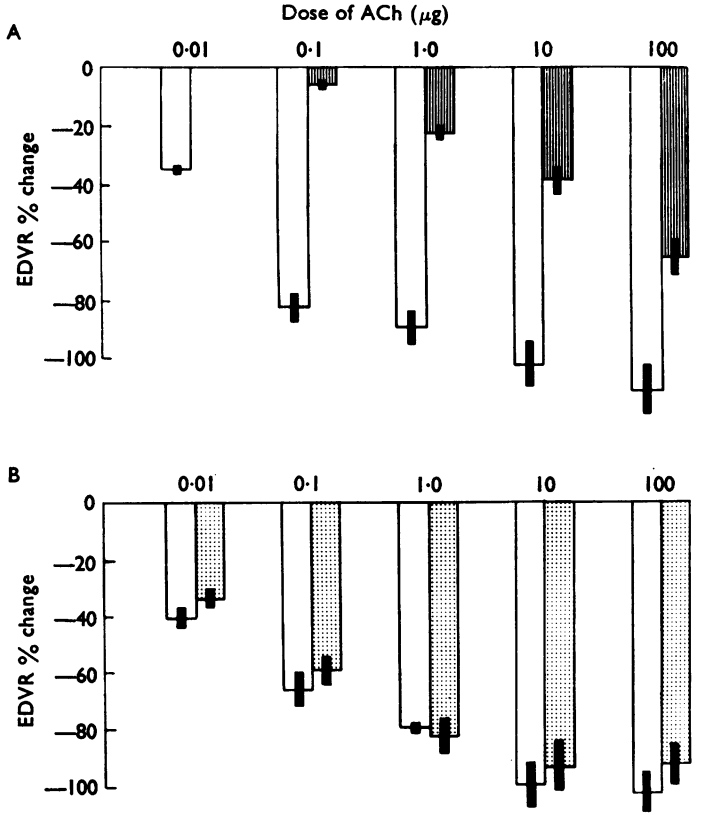


FIG. 6. Effect of atropine (▥, panel A) and propranolol (▨, panel B) on the coronary dilator response to ACh. □, Control; black bars are the standard errors of the means.

dogs showed an increase in contractile force with doses of 1 $\mu$g or more and that the incidence of a "double" positive inotropic effect increases with dose. Thus no animal showed this type of response with doses of 0·1 $\mu$g or less, whereas it was present in twelve of sixteen dogs with a dose of 100 $\mu$g.

### *Arterial pressure and heart rate changes*

Intracoronary ACh in doses of 1 $\mu$g or less produced no changes in arterial pressure. The 10 $\mu$g dose reduced systolic pressure by $11 \pm 3$ mm Hg (mean change $\pm$S.E.) and diastolic pressure by $18 \pm 4$ mm Hg in seventeen dogs. The 100 $\mu$g dose reduced systolic and diastolic pressure by $24 \pm 3$ and $43 \pm 5$ mm Hg respectively in thirteen dogs.

No changes in heart rate were produced in any animal by any of the doses used.

### *Effects of acetylcholine after atropine*

In dogs pretreated with intravenous atropine 0·5 mg/kg, the coronary vasodilator response to ACh was abolished or reduced (Fig. 6A). In addition, the instantaneous coronary flow curves following ACh showed differences before and after atropine. Before atropine, systolic flow was always increased during the first 30 s of the response. After atropine, systolic flow was increased for only 4–6 s. Subsequently, flow decreased and backflow developed in association with an enhanced and accelerated positive inotropic response (Fig. 7).

The effect of atropine on the inotropic responses to ACh is shown in Fig. 8. The negative inotropic response was invariably abolished. The second positive response to small doses of ACh was also abolished and the effect of large doses was significantly reduced. On the other hand, the first peak was moderately increased for the

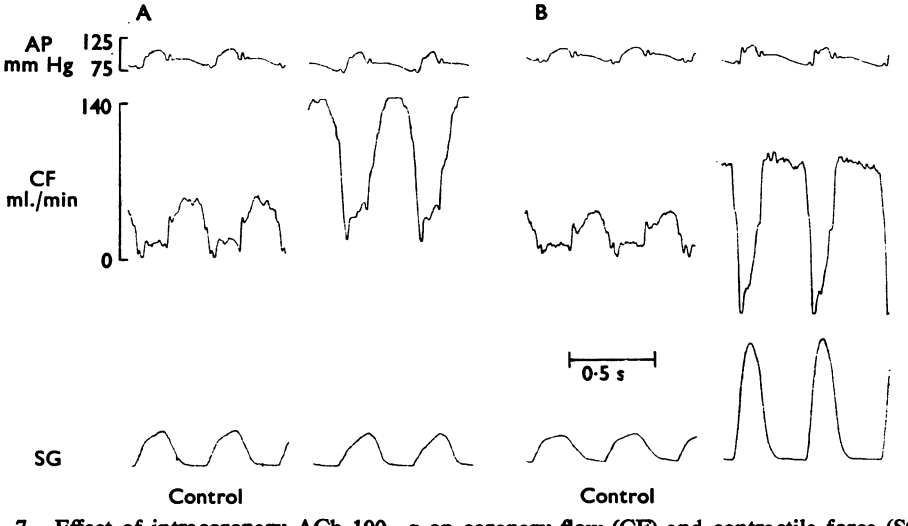


FIG. 7. Effect of intracoronary ACh 100 $\mu$g on coronary flow (CF) and contractile force (SG) before (A) and after (B) atropine. Before atropine ACh produces a small decrease in contractile force and a large increase in both diastolic and systolic flow. After intravenous atropine 0·5 mg/kg, ACh produces a large increase in contractile force; coronary flow still increases but with a very different pattern. Second record in both A and B taken 8 s after the injection. AP, arterial pressure.

10 μg dose and greatly increased for the 100 μg dose (Fig. 9A and B). Pretreatment with atropine caused this initial positive inotropic effect of ACh to begin earlier than before. It commenced at the same time as the coronary flow increase—2–4 s after injection—and reached a peak 5–10 s later.

No changes in arterial pressure were produced by ACh after atropine.

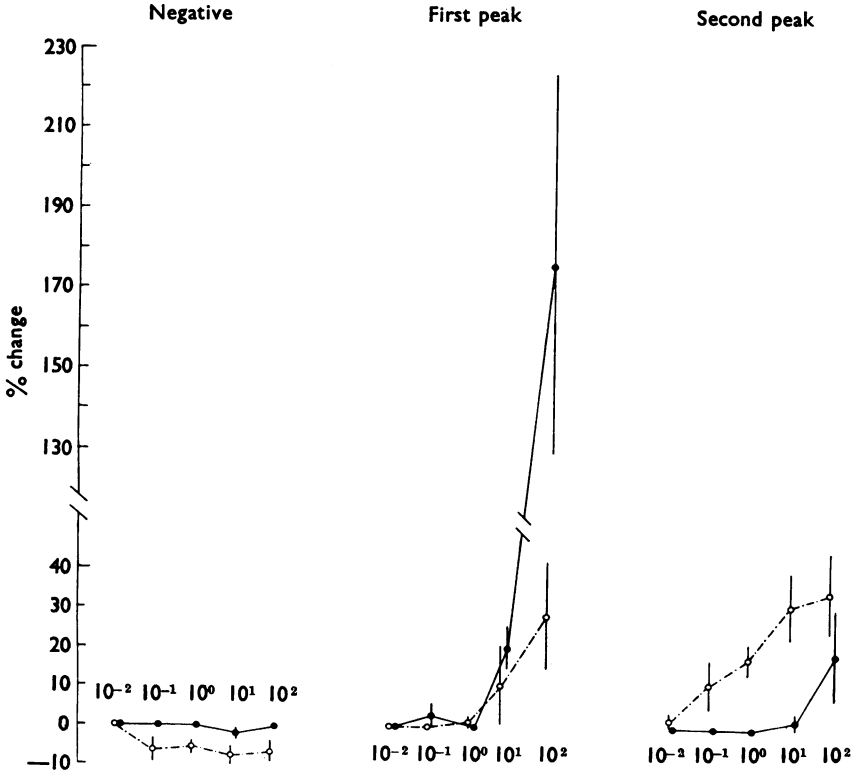


FIG. 8. Effect of atropine on the dose-response relationship of the different inotropic responses to ACh. Means and standard errors are shown. Each point represents the mean of at least five experiments. The negative (left panel) and late positive (right panel) responses are blocked, whereas the early positive inotropic response (centre panel) of large doses of ACh is potentiated. ○—·—○, Control; ●——●, after blockade. Abscissa, dose of ACh (μg); ordinate, % change from control. Note the break in the ordinate scale.

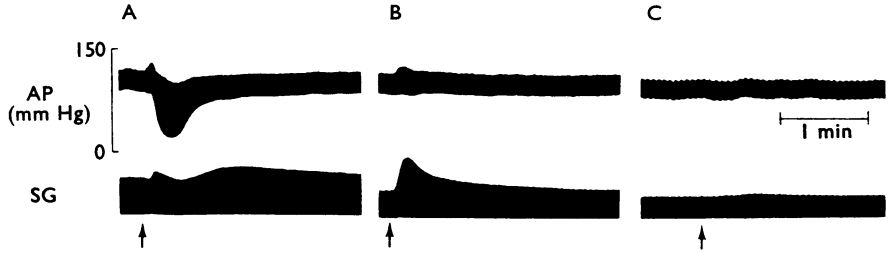


FIG. 9. Effect of 100 μg ACh on contractile force (SG) and aortic pressure (AP) before (A) and after intravenous atropine 0·5 mg/kg (B). Atropine potentiates the early positive inotropic response and reduces the late positive response. After intravenous propranolol 0·5 mg/kg (C), the augmented early positive inotropic response is abolished.

### *Effect of acetylcholine after propranolol*

The effects of intravenous propranolol, 0·25–1·0 mg/kg, on the responses to ACh were observed in eight dogs. The dose of propranolol was adjusted to block the cardiac effects of a dose of intracoronary isoprenaline which produced a response at least as large as the response to ACh.

The effect on the inotropic responses to ACh is shown in Fig. 10. The negative inotropic response was not blocked. The first inotropic peak was greatly reduced and in most cases abolished. The second inotropic peak was unaffected (Fig. 11). The changes in arterial pressure and coronary flow induced by ACh were unaltered by propranolol (Fig. 6B).

### *Effect of combined blockade of cholinergic and adrenergic receptors*

Intravenous propranolol 0·5 μg/kg was given to five of the six animals which had previously received atropine. Propranolol blocked the enhanced first positive inotropic action of ACh. In these five dogs with combined adrenergic and cholinergic block, ACh produced no changes in either coronary blood flow or contractility in doses up to 10 μg (Fig. 9). When the dose was increased to 100 μg, two dogs showed a negative and second positive inotropic response, whereas the remaining three animals showed no response whatever.

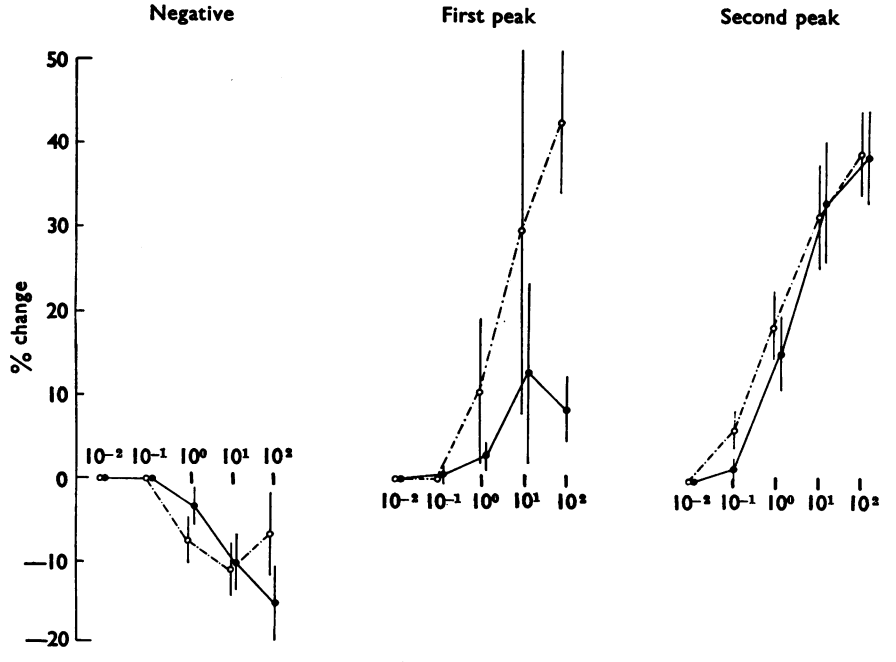


FIG. 10. Effect of propranolol on the dose-response relationship of the inotropic responses to ACh. ○—·—○, Control; ●——●, after blockade. Note that only the early positive response is markedly reduced (centre panel) while the negative (left panel) and late positive (right panel) responses are not modified.

Atropine was given to six of the eight dogs which had previously received propranolol. Before atropine these dogs showed a normal coronary dilator response to ACh, but only a negative and late positive inotropic response. After atropine all these responses were completely blocked for doses up to 10 $\mu$g. The 100 $\mu$g dose was also completely blocked in two dogs and partially blocked in the remaining four.

## Discussion

Our study confirms the potent coronary vasodilator action of ACh that has been described previously by several investigators (Blumenthal *et al.*, 1968; Levy & Zieske, 1969; Denison & Green, 1958; Schreiner, Berglund, Borst & Monroe, 1957). Our finding that the threshold dose for this effect is lower than that for the myocardial response is in agreement with the observations of Blumenthal *et al.* (1968). The dilator response to small doses of ACh appears to be a typical "muscarinic"

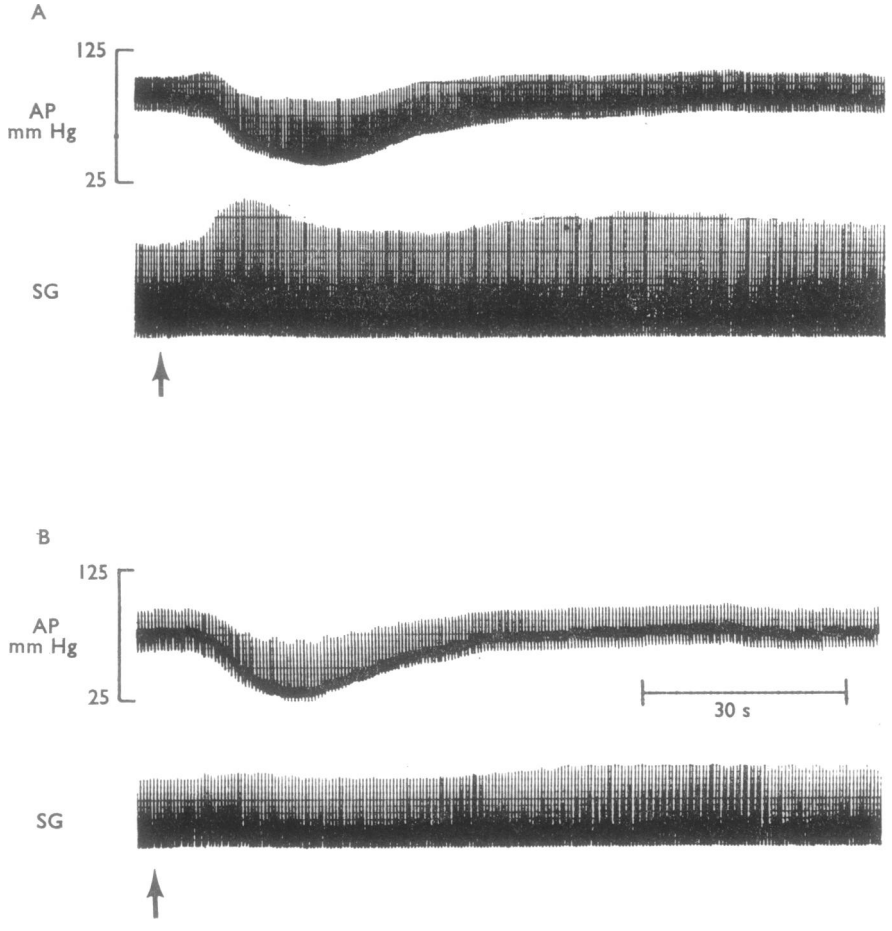


FIG. 11. Effect of propranolol on the inotropic responses to ACh. Note the large decrease in the early positive inotropic response to 100 $\mu$g ACh after propranolol (panel B) with respect to the control (panel A). AP, Arterial pressure; SG, contractile force.

effect and is blocked by atropine, but not by propranolol. With large doses (10–100 $\mu$g of ACh) the response was more complex and resembled that of catecholamine injection (Gaal, Kattus, Kolin & Ross, 1966).

The magnitude of the negative inotropic effect of ACh was small and it was absent in four of our twenty dogs. These observations, obtained in the intact heart, agree with those of Buccino *et al.* (1966), obtained in isolated papillary muscle, that the predominant effect of ACh on ventricular myocardium is a stimulation rather than a depression of contractile force. The flat dose-response curve of the negative inotropic response is of interest. Middleton *et al.* (1956) have reported that the depressor response of isolated papillary muscle was not regularly dependent on the concentration of ACh. The only other paper in which the dose of ACh is related to the negative inotropic effect of the drug is that of Levy & Zieske (1969), who found that the reduction in left ventricular systolic pressure in a paced isovolumetric left ventricular preparation increased with increasing dose over the range 20–50 $\mu$g/l. The differences between these results and our own are not easy to understand, but the differences in preparation, method of determining ventricular myocardial performance and mode of drug administration are so great that any meaningful comparison is not really possible.

In a recent study of the cholinergic responses of isolated cat papillary muscle Buccino *et al.* (1966) observed that the positive inotropic responses to ACh were practically identical in myocardium from normal and catecholamine-depleted hearts and that these responses, unlike those produced by catecholamines, were not associated with a reduction in the time to peak tension. They therefore suggested that there was no need to invoke the hypothesis that acetylcholine releases catecholamines in order to explain its positive inotropic effect. They speculated that even if noradrenaline release did occur it might be in amounts so small as to play little or no role in the positive inotropic response. Alternatively they suggested that noradrenaline release might be the consequence rather than the cause of the stimulatory effect of acetylcholine. We agree with Buccino *et al.* (1966) that catecholamines play no role in the positive inotropic response to small doses of acetylcholine. The initial inotropic effect of larger doses in our dogs, however, was identical to that produced by catecholamines and was blocked by propranolol. It must therefore be concluded that some aspects of the experimental conditions of Buccino *et al.* (1966), for example, isolated tissue, low temperature (30° C) or low stimulation rate (12/min), prevented catecholamine release. The ability of acetylcholine to cause such a release under suitable conditions is well documented both in the heart (Angelakos & Bloomquist, 1965 ; Haeusler, Thoenen, Haefely & Huerlimann, 1968) and in other tissues (Burn & Rand, 1960, 1965 ; Ferry, 1966). In our experiments the first part of the positive inotropic response to acetylcholine was enhanced by atropine. Buccino *et al.* (1966), who observed an enhancement of the positive inotropic response of isolated cat papillary muscle to acetylcholine by atropine, reported that noradrenaline depletion abolished this enhancement. The mechanism of this effect has been clarified by the recent observation that pretreatment with atropine increases the quantity of noradrenaline release by acetylcholine from the isolated cat heart (Haeusler *et al.*, 1968). No definitive evidence is available concerning the site of catecholamine release. The dog ventricle does not contain adrenergic ganglion cells or chromaffin tissue and it therefore seems likely that the release occurs from adrenergic nerve terminals. Dopamine, which has similar cardiac effects to nor-

adrenaline (Brooks, Stein, Matson & Hyland, 1969), has been reported to be present in cardiac mast cells (Jacobowitz, 1967), but the effects of acetylcholine on these cells is unknown.

Buccino *et al.* (1966) reported that atropine did not block the positive inotropic effect of acetylcholine on isolated papillary muscles. Because of this and the relatively large doses of acetylcholine required to produce the response they suggested that a nonspecific membrane effect rather than a drug-receptor interaction might be involved. In a later study (Friedman, Buccino, Sonnenblick & Braunwald, 1967) the same group reported that the positive inotropic effect of acetylcholine on papillary muscle was abolished by raising the stimulus rate from 12/min to above 42/min. The situation seems to be quite different in the intact heart. In our dogs with an average heart rate of 150/min and in the studies of Hollenberg, Carriere & Barger (1965), with a similar rate, acetylcholine consistently produced a positive inotropic response which was abolished by atropine. The positive inotropic effect observed by Hollenberg *et al.* (1965) occurred after stopping intracoronary infusions and was referred to by them as acetylcholine " rebound ". The late positive inotropic effect (the " second peak " produced by rapid injections of ACh in our preparations) is probably analogous and our observation that propranolol does not block this effect is consistent with their views that the rebound is not dependent upon catecholamine release. The response appears to involve a muscarinic receptor, for it is blocked by atropine. The delay in the response as compared with the negative inotropic effect may indicate a longer series of intermediate reactions between drug-receptor combination and the effector response. Alternatively both negative and positive effects may begin concurrently but with different time courses, so that the negative effect predominates initially but is overcome by the positive effect later in the response.

In summary, our observations indicate that in the beating heart *in situ* the positive inotropic effect of acetylcholine involves two mechanisms. One mechanism, which appears to be catecholamine dependent, is seen only with large doses, is enhanced by atropine and is blocked by propranolol. It reaches a maximum within 18 s of rapid intracoronary injection and is characterized by a reduction in the time to peak tension. The second mechanism has a lower threshold and the time to peak tension is unchanged. It is not affected by propranolol but is blocked by atropine. When acetylcholine is given by rapid intracoronary injection the peak of the response occurs later than the catecholamine-dependent peak. Our results indicate that neither non-muscarinic cholinergic receptors nor a nonspecific membrane effect of ACh, which may play a role in the positive inotropic response of isolated myocardium under certain conditions (Buccino *et al.*, 1966), participate in the cholinergic inotropic responses of the heart *in situ*.

M. I. B. is a postdoctoral fellow supported by U.S.P.H.S. Grant HE 5696. The study was aided by a grant from the American Medical Association Education and Research Foundation and was performed during the tenure by G. R. of an Established Investigatorship of the American Heart Association. The authors thank Miss Madeline Kurrasch for able technical assistance.

## REFERENCES

ANGELAKOS, E. T. & BLOOMQUIST, E. (1965). Release of norepinephrine from isolated hearts by acetylcholine. *Archs int. Physiol. Biochem.*, **73**, 397–402.

BLUMENTHAL, M. R., WANG, H., MARKEE, S. & WANG, S. C. (1968). Effects of acetylcholine on the heart. *Am. J. Physiol.*, **214**, 1280–1287.

BROOKS, H. L., STEIN, P. D., MATSON, J. L. & HYLAND, J. W. (1969). Dopamine-induced alterations in coronary hemodynamics in dogs. *Circulation Res.*, **24**, 699–897.

BUCCINO, R. A., SONNENBLICK, E. H., COOPER, T. & BRAUNWALD, E. (1966). Direct positive inotropic effect of acetylcholine on myocardium. *Circulation Res.*, **19**, 1097–1108.

BURN, J. H. & RAND, M. J. (1960). Sympathetic postganglionic cholinergic fibres. *Brit. J. Pharmac. Chemother.*, **15**, 56–66.

BURN, J. H. & RAND, M. J. (1965). Acetylcholine in adrenergic transmission. *Ann. Rev. Pharmac.*, **5**, 163–182.

DENISON, A. B. & GREEN, H. (1958). Effects of autonomic nerves and their mediators on the coronary circulation and myocardial contraction. *Circulation Res.*, **7**, 633–643.

ELIAKIM, M., BELLET, S., TAWIL, E. & MULLER, D. (1961). Effect of vagal stimulation and acetylcholine on the ventricle. *Circulation Res.*, **9**, 1372–1379.

FERRY, C. B. (1966). Cholinergic link hypothesis in adrenergic neuroeffector transmission. *Physiol. Rev.*, **46**, 420–455.

FRIEDMAN, W. F., BUCCINO, R. A., SONNENBLICK, E. H. & BRAUNWALD, E. (1967). Effects of frequency of contraction and ionic environment on the responses of heart muscle to acetylcholine. *Circulation Res.*, **21**, 573–582.

GAAL, P. G., KATTUS, A. A., KOLIN, A. & ROSS, G. (1966). Effects of adrenaline and noradrenaline on coronary blood flow before and after beta adrenergic blockade. *Br. J. Pharmac. Chemother.*, **26**, 713–722.

HAEUSLER, G., THOENEN, H., HAEFELY, W. & HUERLIMANN, A. (1968). Electrical events in cardiac adrenergic nerves and noradrenaline release from the heart induced by acetylcholine and KCl. *Naunyn-Schmiedeberg's Arch. Pharmak. exp. Path.*, **261**, 389–411.

HOLLENBERG, M., CARRIERE, S. & BARGER, A. C. (1965). Biphasic action of acetylcholine on ventricular myocardium. *Circulation Res.*, **16**, 527–536.

JACOBOWITZ, D. (1967). Histochemical studies of the chromaffin cells and adrenergic nerve fibers to the cardiac ganglia of several species. *J. Pharmac. exp. Ther.*, **158**, 227–240.

LEVY, M. N. & ZIESKE, H. (1969). Comparison of the cardiac effects of vagus nerve stimulation and of acetylcholine infusions. *Am. J. Physiol.*, **216**, 890–897.

MIDDLETON, S., OBERTI, C., PRAGER, R. & MIDDLETON, H. H. (1956). Stimulating effect of acetylcholine on the papillary myocardium. *Acta physiol. latinoam.*, **6**, 82–89.

SCHREINER, G. L., BERGLUND, E., BORST, H. G. & MONROE, R. G. (1957). Effect of vagus stimulation and of acetylcholine on myocardial contractility, $O_2$ consumption and coronary flow in dogs. *Circulation Res.*, **5**, 562–567.

(*Received October 9*, 1969)