Br. J. Pharmac. (1973), **48**, 612–619.

Effects of intracoronary infusions of acetylcholine and nicotine on the dog heart *in vivo*

G. ROSS

Department of Physiology, UCLA School of Medicine, Los Angeles, California 90024, U.S.A.

Summary

1. In anaesthetized dogs intracoronary infusions of high doses of nicotine and acetylcholine increased myocardial contractile force and this could be prevented by pre-treatment with desmethylimipramine or phenoxybenzamine.
2. The inotropic effect of nicotine was brief and subsided during the continuing infusion of the drug. The infusion of nicotine did not reduce the inotropic effects of cardiac sympathetic nerve stimulation.
3. The inotropic effect of intracoronary acetylcholine often fluctuated during prolonged infusions and was not altered by pretreatment with atropine. Acetylcholine infusions reduced the inotropic responses produced by cardiac sympathetic nerve stimulation and led to a substantial transient reduction in the associated pressor responses. Intracoronary acetylcholine also reduced the pressor and inotropic effect of intravenous noradrenaline. The attenuation of these adrenergic cardiovascular responses by acetylcholine was prevented by atropine.

Introduction

Acetylcholine has complex effects on cardiac tissues. Small doses have negative inotropic and chronotropic actions, antagonize the effects of infused noradrenaline (Hollenberg, Carriere & Barger, 1965) and reduce the noradrenaline output evoked by cardiac sympathetic nerve stimulation (Löffelholz & Muscholl, 1969). Larger doses produce effects resembling those of nicotine and release noradrenaline from sympathetic nerve terminals, thereby increasing myocardial contractile force (Angelakos & Bloomquist, 1965 ; Löffelholz, 1967 ; Dempsey & Cooper, 1969).

Most of the published reports have described the effects of acetylcholine and nicotine on isolated hearts perfused with saline solutions, commonly at low temperature and perfusion pressure. The purpose of the present investigation was to study the characteristics of the myocardial responses to infusions of catecholamine-releasing doses of acetylcholine and nicotine in the dog heart *in situ* and to determine the effects of these agents on the response to cardiac sympathetic nerve stimulation and to intravenous noradrenaline.

Methods

Twenty-three dogs were anaesthetized with intravenous chloralose 100 mg/kg following pre-medication with intramuscular morphine sulphate 5 mg/kg. The trachea was intubated and ventilation was maintained with a Bird positive pressure ventilator delivering a 40% oxygen in air mixture. After opening the chest in the fifth left intercostal space, the pericardium was incised and the anterior descending

coronary artery was dissected free from the myocardium over a distance of 2–3 cm. Drugs were infused into this vessel by a Harvard infusion pump via an indwelling 30 gauge needle. This needle had no effect on coronary flow. Myocardial force was determined by a Walton-Brodie strain gauge arch sewn onto the myocardium downstream from the needle. In several animals another arch serving as a control was applied upstream from the needle.

The left ansa subclavia was dissected and cut. The distal end was placed over bipolar platinum electrodes and stimulated with rectangular pulses of 8 V, 1 ms duration, with a frequency of 10 Hz for periods of 30–90 seconds.

Aortic pressure was determined with a Statham P23Db transducer via a polyethylene catheter inserted into a carotid artery and advanced into the aortic arch. Blood flow was measured in the anterior descending coronary artery (5 dogs) with a Biotronex 610 electromagnetic flowmeter using cuff-type probes. The zero reference was determined by mechanically occluding the vessel downstream from its probe. Calibration was performed by placing the probe on a branch of the femoral artery and withdrawing blood from the vessel at a constant rate with the Harvard pump. All variables were recorded on a Beckman S11 Dynograph.

The drugs used were acetylcholine chloride, nicotine bitartrate, atropine sulphate, desmethylimipramine hydrochloride, phenoxybenzamine hydrochloride and (−)-noradrenaline bitartrate. Their concentrations are given in terms of the salts. The drugs were diluted in physiological saline solution and the maximum volume infusion rate was 0·5 ml/minute. When multiple infusion of either acetylcholine or nicotine was carried out in the same animal, the interval between infusions was at least 20 minutes. The administration of either agent did not modify the subsequent response to the other. Statistical inference was made by Students '*t*' test for paired comparisons.

Results

Effects of intracoronary nicotine

Nicotine was infused into the anterior descending coronary arteries of nine dogs at rates of 100 μg/min and 200 μg/minute. Three dogs developed multiple ectopic beats within 1–2 min and the responses of these animals were not analysed. The remaining six showed no statistically significant change in aortic pressure or heart rate. Contractile force increased in the area perfused with nicotine but not in other areas. The increase reached its maximum within 1–2 min of onset and then declined to preinfusion values during the next 2–5 min despite the continued administration of the drug. Similar changes have been observed in the isolated rabbit heart by Löffelholz (1970a) and in accordance with his terminology the

TABLE 1. *Maximum percent increases in contractile force (means ± standard error) produced by intracoronary infusions of nicotine (6 dogs) and of acetylcholine (7 dogs) before and after intravenous atropine (0·1 mg/kg).*

Agent	Infusion rate (μg/min)	Percent contractile force increase	
		Before atropine	After atropine
Nicotine	100	70±15	94±18
	200	111±35	159± 39
Acetylcholine	100	38±8	56±9
	200	70±26	100±27

period of declining response will be referred to as the period of 'autoinhibition'. Pretreatment with atropine 0·1 mg/kg i.v. did not significantly ($P>0·05$) alter the amplitude of the inotropic response (Table 1).

Stimulation of the left cardiac sympathetic nerves (CSN) during the period of autoinhibition produced changes in pressure, rate and contractile force which did not differ significantly ($P>0·05$) from those produced by stimulation in the absence of nicotine (Table 2). CSN stimulation during the inotropic action of nicotine produced a further increment in contractile force and similar pressure and rate changes to those produced before the nicotine infusion.

TABLE 2. *Effect of stimulation of the left cardiac sympathetic nerves (CSN) on systolic and diastolic arterial pressures (SP, DP, mmHg) and on heart rate (HR) and contractile force (CF).*

	Before nicotine ($n=6$)		During nicotine autoinhibition ($n=6$)	
	Control	CSN	Control	CSN
SP	115±7	153±10	119±9	159±15
DP	81±7	118±6	85±6	123±10
HR	66±4	96±6	70±6	98±8
CF	100	129±7	100	126±7

Contractile force (CF) is expressed as percent of the 'before nicotine' value. Values shown are means ± S.E. The effects of stimulation before nicotine and during nicotine autoinhibition did not differ significantly ($P>0·1$).

Effects of intracoronary acetylcholine

Intracoronary infusions of acetylcholine were administered to 10 dogs. Because the study was particularly concerned with catecholamine-releasing doses, all dogs received infusion rates of 100 μg/min or above although several were also given smaller doses. No arrhythmias occurred. Contractile force increased in the perfused area. The time of onset of this response was variable but the maximum effect was always reached within 2 min and then declined despite the continued administration of the drug. This decline, in contrast to that seen with nicotine, was often irregular, being interrupted with repetitive increases and decreases in six of the ten animals. These large doses of acetylcholine usually produced systemic hypotension and tachycardia. The 200 μg/min infusion rate, for example, caused arterial pressure to fall from a mean value of 120/80 to 110/52 mmHg and heart rate to increase from a mean of 79 to a mean of 140 beats/min in ten dogs. However, as the infusion continued, pressure returned to control levels and the tachycardia lessened. Two or three cycles of hypotension and tachycardia at

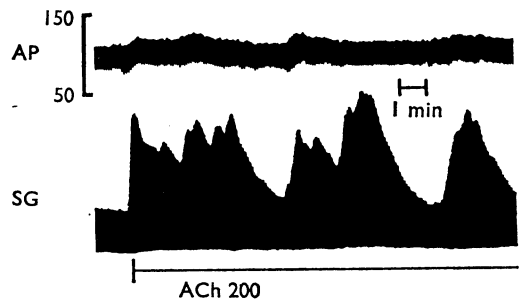

FIG. 1. Fluctuant inotropic response to intracoronary acetylcholine (ACh) 200 μg/min after intravenous atropine 0·1 mg/kg. Upper trace—aortic pressure (AP, mmHg), lower trace contractile force (SG).

intervals of 2–4 min occurred during a single prolonged infusion in some animals before a steady state was reached. Pretreatment with atropine 0·1 mg/kg i.v. abolished the systemic effects of acetylcholine but did not significantly affect the magnitude of the inotropic responses (Table 1) or their fluctuations (Figure 1).

Effects of intracoronary acetylcholine on the cardiovascular response to cardiac sympathetic nerve stimulation

In the absence of drugs CSN stimulation increased arterial pressure, heart rate and contractile force. A slight dip in the pressure record often occurred a few seconds after the onset of stimulation following the initial steep rise in pressure. During the intracoronary infusion of acetylcholine the effect of CSN stimulation on contractile force was abolished or greatly reduced in the area of myocardium perfused by acetylcholine but not in other areas (Figure 2). Indeed a reduction

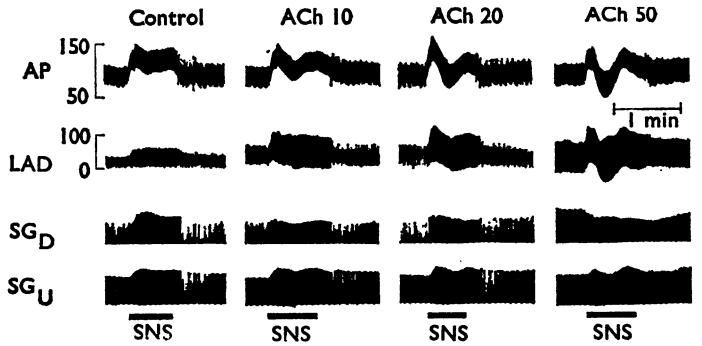

FIG. 2. Effects of increasing intracoronary infusion rates of acetylcholine (ACh, 10, 20 and 50 μg/min) on the responses to a constant stimulus to the left cardiac sympathetic nerves. AP=aortic pressure (mmHg), LAD=anterior descending coronary flow (ml/minute). Note: (1) that sympathetic nerve stimulation (SNS) depresses contractile force in the area perfused by acetylcholine (SG_D) but increases it in other areas (SG_U) and (2) the pressure dip which increases with increasing doses of acetylcholine.

TABLE 3. *Increments in systolic and diastolic pressure (mmHg), heart rate (beats/min) and contractile force (%) during cardiac sympathetic nerve stimulation (CSN) before acetylcholine and during intracoronary acetylcholine* 200 *μg/min before and after intravenous atropine* 0·1 *mg/kg.*

	Systolic pressure				Diastolic pressure							
		Increment				Increment			Heart rate		Cont. force	
	Prestim value	Init.	Dip	Final	Prestim value	Init.	Dip	Final	Prestim value	Inc.	Prestim value	Inc.
CSN before ACh (n=10)	127±4	43±5	39±5	53±6	82±4	33±5	28±6	43±5	71±9	35±6	100	37±12
CSN during ACh (n=10)	121±5	30±3	17±3	38±8	81±5	19±3	−8±4	27±3	100±12	51±8	125±9	−5±8
CSN after Atrop. (n=7)	122±8	29±6	24±7	33±7	91±8	17±4	13±5	23±9	206±10	20±8	100	42±19
CSN during ACh after Atrop. (n=7)	123±6	21±3	17±4	33±6	92±6	15±2	2±6	26±6	215±12	25±10	128±12	20±11

Values are means ± S.E. Pressure was measured at its maximum level before the dip (Init), at the lowest point of the dip (Dip) and at the end of a 30 s stimulation (Final).

of force often occurred at the same time as force in the non-perfused area was increasing (Figure 2). The systemic response to CSN stimulation was also modified by intracoronary acetylcholine (Fig. 2 Table 3). The increase in heart rate was greater ($P<0.05$), the initial rise in both systolic and diastolic pressure was diminished ($P<0.001$) and the subsequent dip was accentuated ($P<0.01$) often to the extent that pressure transiently fell below pre-stimulation values. The magnitude of this dip was dependent both on the infusion rate (Fig. 2) of acetylcholine and on the intensity of nerve stimulaion. Coronary flow was increased by acetylcholine in all five dogs in which this variable was measured and increased further during CSN stimulation (Figure 2). Although atropine 0·1 mg/kg i.v. itself reduced the cardiovascular effects of CSN stimulation it also reduced or abolished the effects of acetylcholine infusion on the responses to CSN stimulation (Table 3).

Effects of intracoronary acetylcholine on the cardiovascular response to noradrenaline

Noradrenaline 5–10 μg was administered by rapid intravenous injection before and during intracoronary infusions of acetylcholine 200 μg/minute. The pressor and inotropic actions of noradrenaline were attenuated by acetylcholine and this attenuation was prevented or reduced by the prior administration of atropine 0·1 mg/kg i.v. (Table 4).

TABLE 4. *Changes in aortic pressure (AoP) and contractile force produced by intravenous noradrenaline (NA) in 5 dogs before and during the intracoronary infusion of acetylcholine* 200 μg/*minute.*

Dog No.	NA dose	Intravenous NA before ACh AoP before NA	AoP after NA	Increase in cont. force	Intravenous NA during Ach AoP before NA	AoP after NA	Increase in cont. force
1	5	90/60	165/130	131	95/60	135/100	67
2	10	125/100	195/155	50	110/80	140/110	31
2 (atropine)	10	110/85	210/190	100	105/85	210/190	88
3	10	135/105	220/165	114	140/105	175/130	13
3 (atropine)	10	135/105	220/165	114	130/105	240/200	143
4	5	125/80	160/110	45	130/85	155/105	18
4 (atropine)	5	115/80	195/165	150	115/85	185/160	133
5	10	150/90	220/175	93	155/90	200/160	14

The responses were re-tested after intravenous atropine 0·1 mg/kg in three of the dogs.

Effects of desmethylimipramine and phenoxybenzamine on the myocardial responses to intracoronary nicotine and acetylcholine

Desmethylimipramine was injected into the coronary arteries of six atropinized dogs in doses of 0·3 mg repeated at 3–6 min intervals and the contractile force responses to intracoronary acetylcholine 200 μg/min and nicotine 200 μg/min were tested after a total dose of 1·2 mg. Desmethylimipramine itself produced no significant change in aortic pressure or heart rate but usually caused a transient slight reduction in force which recovered within 2–3 minutes. The inotropic effects of both nicotine and acetylcholine were completely blocked in four of the dogs and increasing the dose of desmethylimipramine to 9 mg blocked the remaining two.

A similar procedure was followed with phenoxybenzamine which was injected in repeated doses of 10 mg into the coronary arteries of seven dogs which had been pretreated with atropine 0·1 mg/kg. The inotropic responses to both acetylcholine and nicotine were blocked by 40 mg phenoxybenzamine in 3 dogs and increasing the dose to 80 mg blocked the remainder.

Discussion

These experiments have shown that continuous intracoronary nicotine infusions produce only transient inotropic effects on the dog heart *in situ* and that cardiovascular responses to CSN stimulation before and during nicotine infusion did not differ significantly. This increased force is presumably due to noradrenaline release from intramyocardial sympathetic nerve terminals (Burn & Rand, 1958). Löffelholz (1970a) found that the noradrenaline output from isolated perfused rabbit hearts infused with nicotine decayed exponentially with time and was not detectable after 2–4 minutes. He suggested that nicotinic agents produce an explosive release of noradrenaline which is terminated within a few seconds by a process which he termed 'autoinhibition'. In other experiments the noradrenaline output evoked by cardiac sympathetic nerve stimulation was not altered by nicotine infusions (Löffelholz, 1970b). Present experiments suggest that nicotine has similar effects in the intact dog heart.

Intracoronary infusions of acetylcholine also increased myocardial force. Allowing for the rapid destruction of acetylcholine in blood and the antagonism by acetylcholine of the action of noradrenaline on the myocardium (Hollenberg *e al.*, 1965 ; Jacob, Miller & Gilmore, 1971) it seems that the positive inotropic potency of acetylcholine is about equal to nicotine in the normally perfused dog heart. However, the output of noradrenaline induced by acetylcholine in the isolated rabbit heart (Löffelholz, 1970a) in the absence of atropine was very much smaller than that produced by similar concentrations of nicotine ; atropine increased the release of noradrenaline by acetylcholine ten-fold but did not increase the output of noradrenaline by nicotinic agents (Lindmar, Löffelholz & Muscholl, 1968). They concluded that the peripheral adrenergic nerve fibre contained inhibitory muscarinic receptors and that acetylcholine could produce a substantial noradrenaline release only if its muscarinic action was blocked in the intact animal. It is not easy to relate these findings to the present study because large doses of acetylcholine infused into the coronary arteries produce a direct negative inotropic effect, a positive inotropic action due to catecholamine release, hypotension and tachycardia. The hypotension was probably due to peripheral vasodilatation and the tachycardia may have been the result of diminished baroreceptor activity during the hypotensive phase. The possibility arises, therefore, that part of the inotropic response to intracoronary administration of acetylcholine was the result of reflex effects. However, the contribution of reflex inotropic stimulation to the total response was probably small because (1) inotropic responses in areas of the myocardium upstream from the point of acetylcholine infusion were negligible (Fig. 2), (2) inotropic effects were seen after atropine which abolished the hypotensive effect of acetylcholine (Figs. 1 and 3) sympathetic nerve stimulation is ineffective in producing positive inotropic effects in areas perfused by acetylcholine (Fig. 2 Table 3). It seems likely, therefore, that the inotropic effect of acetylcholine is mainly due to a direct action on the post-ganglionic nerve endings. The similarity of the inotropic responses to acetylcholine and nicotine and the fact that atropine did not significantly potentiate the inotropic action of acetylcholine indirectly suggest that muscarinic inhibition of noradrenaline release by acetylcholine is small in the myocardium *in situ.*

The inotropic effect of acetylcholine, unlike that of nicotine, fluctuated considerably during a single infusion. These fluctuations could not be ascribed to indirect

effects resulting from arterial pressure changes because they persisted after atropine had abolished the pressure fluctuations (Figure 1). A possible explanation is that acetylcholine produces an intermittent release of noradrenaline implying a fluctuation in the level of 'autoinhibition'. However, no direct evidence bearing on this point is available.

Kent, Dempsey & Cooper (1970) have shown that the positive inotropic response produced by acetylcholine in isolated cat papillary muscles is blocked by cocaine. Furthermore, the release of noradrenaline by nicotine from the isolated cat pulmonary artery is blocked by cocaine, desmethylimipramine and phenoxybenzamine (Su & Bevan, 1970). These agents inhibit the neuronal uptake of noradrenaline and Su & Bevan (1970) therefore suggest that the release of noradrenaline by nicotinic drugs may require an intact noradrenaline uptake mechanism. The present experiments showing that desmethylimipramine and phenoxybenzamine blocked the inotropic effects of both nicotine and acetylcholine support this view.

Acetylcholine infusions blocked the inotropic effect of CSN stimulation but failed to do so after atropine. A muscarinic inhibition of the inotropic response to exogenous noradrenaline has been reported in many species (Hollenberg *et al.*, 1965; Dempsey & Cooper, 1968; Jacob *et al.*, 1971) and was also demonstrated in the present experiments (Table 4). Acetylcholine also produced a muscarinic inhibition of the noradrenaline output evoked by stimulating the sympathetic nerves of the isolated perfused rabbit heart (Löffelholz, 1970a). The cholinergic block of the inotropic response to CSN stimulation in the dog heart *in situ* may have been due to a combination of diminished noradrenaline release and reduced myocardial response.

The reduction in contractile force often observed when the cardiac sympathetic nerves were stimulated during an intracoronary acetylcholine infusion (Fig. 2) was an unexpected finding. It was not due to inadequate coronary flow as this was substantially increased above cntrol levels (Figure 2). Szentivanyi, Pace, Wechsler & Randall (1967) have shown that stimulation of individual cardiac sympathetic nerves may increase force in one area of myocardium and reduce it in an adjacent area. This effect was sometimes observed in the present study in the absence of drugs. Conceivably, the increased contractile force induced by nerve stimulation in the area not perfused by acetylcholine interferes with force development in the adjacent perfused area where the sympathetic inotropic effect is blocked.

One of the most striking observations in the present study was the transient drop in pressure which occurred shortly after the onset of the pressor response induced by CSN stimulation during the infusion of acetylcholine (Figure 2). At present no explanation can be given for this unusual response.

This study was supported by a grant from the American Medical Association Education Research Foundation and was performed during the tenure of an Established Investigatorship of the American Heart Association.

REFERENCES

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

BURN, J. H. & RAND, M. J. (1958). Action of nicotine on the heart. *Brit. Med. J.*, **1**, 137–139.

DEMPSEY, P. J. & COOPER, T. (1969). Ventricular cholinergic receptor systems: interaction with adrenergic systems. *J. Pharmac. exp. Ther.*, **167**, 282–290.

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

JACOB, R., MILLER, D. T. & GILMORE, J. P. (1971). Antiadrenerge Effekte von Acetylcholin und ihre Bedeutung für Kontraktionskraft und Aktionspotential des Saugerventrikelmyokards. *Arzneimittel-Forsch.*, **25**, 141–153.

KENT, K. M., DEMPSEY, P. J. & COOPER, T. (1970). Cocaine blockade of the acetylcholine positive inotropic response in ventricular myocerdium. *Proc. Soc. exp. Biol. Med.*, **135**, 292–296.

LINDMAR, R., LOFFELHOLZ, K. & MUSCHOLL, E. (1968). A muscarinic mechanism inhibiting the release of noradrenaline from peripheral adrenergic nerve fibres by nicotinic agents. *Br. J. Pharmac. Chemother.*, **32**, 280–294.

LÖFFELHOLZ, K. (1967). Untersuchungen über die Noradrenalin-Freisetzung durch Acetylcholin am perfundierten Kaninchenherzen. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **258**, 108–122.

LÖFFELHOLZ, K. (1970a). Autoinhibition of nicotinic release of noradrenaline from postganglionic sympathetic nerves. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **267**, 49–63.

LÖFFELHOLZ, K. (1970b). Nicotinic drugs and postganglionic sympathetic transmission. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **267**, 64–73.

LÖFFELHOLZ, K. & MUSCHOLL, E. (1969). A muscarinic inhibition of the noradrenaline release evoked by postganglionic sympathetic nerve stimulation. *Naunyn-Schmiedeberg's Arch. Pharmak*, **265**, 1–15.

SZENTIVANYI, M., PACE, J. B., WECHSLER, J. S. & RANDALL, W. C. (1967). Localised myocardial responses to stimulation of cardiac sympathetic nerves. *Circulation Res.*, **21**, 691–702.

SU, C. & BEVAN, J. A. (1970). Blockade of the nicotine-induced norepinephrine release by cocaine, phenoxybenzamine and desipramine. *J. Pharmac exp. Ther.*, **175**, 533–540.

(*Received February* 26, 1973)