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CORONARY VASOCONSTRICTION BY LOCALLY ADMINISTERED ACETYLCHOLINE, CARBACHOL AND BETHANECHOL IN ISOLATED, DONOR-PERFUSED, RAT HEARTS

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1 Experiments were carried out on rat isolated heart preparations in which the coronary vasculature was perfused through the aorta at a constant flow rate with arterial blood from donor animals. Single doses of drugs were injected into the aortic cannula.

2 Small doses of acetylcholine, carbachol or bethanechol decreased perfusion pressure (PP) without markedly affecting left ventricular pressure (LVP) and heart rate (HR); larger doses of these drugs increased PP (vasoconstriction), and decreased LVP and HR in a dose-dependent manner.

3 Acetylcholine, carbachol and bethanechol had almost no effects when perfused through the aorta in such a way as to exclude the coronary vessels.

4 Coronary vasoconstriction in response to acetylcholine, carbachol and bethanechol was not significantly affected by reserpine pretreatment, phentolamine or hexamethonium, but was antagonized by small doses of atropine.

5 From these results it is concluded that in the coronary vasculature of the rat, the receptors involved in the vasoconstrictor actions of acetylcholine, carbachol and bethanechol are muscarinic.

Introduction

There have been many studies of the effects of acetylcholine on the coronary circulation (reviewed by Higgins, Vatner & Braunwald, 1973). There is general agreement that acetylcholine, when administered locally into the coronary vessels, is a potent vasodilator (Katz, Lindner, Weinstein, Abramson & Jochim, 1938; Shreiner, Berglund, Borst & Monroe, 1957; Hashimoto, Shigei, Imai, Saito, Yago, Uei & Clark, 1960; Blumental, Wang, Markee & Wang, 1968; Levy & Zieske, 1969; Sakai, Sugano, Taira & Hashimoto, 1974). However, all of these experiments were performed on dog hearts.

The present experiments on rat isolated, blood-perfused heart preparations were undertaken to investigate further the mode of action of locally administered acetylcholine on the coronary vascular bed and to compare it with the effects of two other cholinomimetic drugs, carbachol and bethanechol. In view of the unusual nature of the results obtained, supplementary studies were also carried out on guinea-pig isolated hearts.

Methods

Male rats of the Sprague-Dawley strain and male guinea-pigs of the Hartley strain were anaesthetized with sodium pentobarbitone (65 mg/kg i.p.) and urethane (1.2 g/kg i.p.), respectively.

Coronary perfusion

Rats The right jugular vein and carotid artery of donor rats (600 to 700 g), which were not ventilated artificially, were cannulated with polyethylene cannulae. Heparin sodium (1000 units/kg) was injected into the femoral vein, and the mean systemic blood pressure was measured from the left femoral artery with a pressure transducer (Nihon Kohden, MPU-0.5). The carotid and jugular cannulae were connected to the perfusion circuit after priming with about 15 ml of blood freshly drawn from other heparinized rats.

Recipient rats (about 300 g) were tracheotomized, and positive-pressure respiration with room air initiated with a respirator (Takashima Co.) at a rate of 70/min and a tidal volume of 3 to 5 ml. The chest was opened and the aorta, pulmonary artery, inferior vena cava and both right and left superior venae cavae, were exposed and separated from the surrounding tissues. Both vagi and phrenic nerves were sectioned and heparin sodium (1000 units/kg) injected through the femoral vein. Polyethylene cannulae were introduced into the right ventricle via the right superior vena cava (i.d. 1.5 mm; o.d. 2 mm) and into the arch of the aorta via the innominate artery (i.d. 0.7 mm; o.d. 1.5

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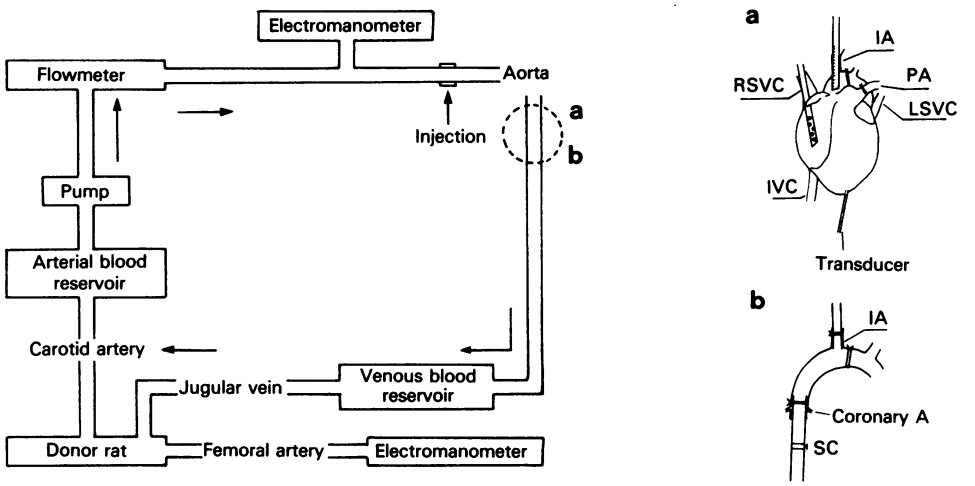

Figure 1 Schematic diagram of the perfusion system for the heart (a) or aortic (b) preparation. RSVC, right superior vena cava; LSVC, left superior vena cava; IVC, inferior vena cava; PA, pulmonary artery; IA, innominate artery; SC, screw clamp.

mm). Artificial ventilation was then stopped and the descending aorta, pulmonary artery, left superior vena cava and inferior vena cava were ligated in that order. The lungs were ligated at the hilus and blood from the right carotid artery of the donor was conducted at a fixed flow rate into the stump of the aorta, and thus into the coronary circulation of the isolated heart, by means of a precalibrated peristaltic pump (Mitsumi Science, SJ-1210). The circulating blood was about 37°C when it entered the perfused heart. Total coronary blood inflow was measured continuously with a square-wave electromagnetic flowmeter (Nihon Kohden, MF-25), the flow rate being precalibrated and re-checked at the end of the experiment. Mean arterial perfusion pressure (PP) was recorded near the aortic cannula with a pressure transducer (Nihon Kohden, MPU-0.5). Left ventricular pressure (LVP) was measured by puncturing the ventricular wall with a 3.2 cm 22 gauge needle which was fixed to the heart with biotissue adhesives (Aron Alpha A, Sankyo) and attached via a polyethylene tube to a pressure transducer (Nihon Kohden, MPU-0.5). Heart rate (HR) was measured with a heart rate counter (Nihon Kohden, AT-600G) and permanent records were made on a Nihon Kohden, WI-680G recorder.

In some of the experiments the heart was paced at 300 beats per min by square-wave impulses (10 V, 1 ms, 5 Hz). This rate was chosen since control rates of below 300 beats per min were observed in spontaneously beating hearts. Pacing was applied by silver-electrodes attached to the auricle and ventricle with an electronic stimulator (DPS-10, DIA Medical System Co.).

The perfusing blood from the carotid cannula of the donor first flowed into a small glass bottle. The volume of blood (about 4 ml) in the bottle (arterial reservoir) was maintained constant by an adjustment with a small screw clamp. The venous outflow from the right ventricle was received by a venous reservoir and returned to the jugular vein of the donor by gravity (15 cm drop of hydrostatic pressure). The donor rat and the isolated heart of the recipient were placed on heated tables; the rectal temperature of the donor rat was maintained at between 36 and 38°C throughout the experiment. All of the experiments were performed on beating hearts; it was difficult to induce a sustained ventricular fibrillation in rat hearts. The perfusion arrangement is schematically illustrated in Figure 1a. The perfusion apparatus was essentially the same as that described in a previous paper (Sakai, 1978).

Myocardial oxygen consumption (MVO_2) was estimated from the arteriovenous (A–V) difference in the oxygen content, and expressed in ml of oxygen consumed per 100 g of the LV wet weight (W) per min: MVO_2 (ml min^{-1} 100 g^{-1} LVW) = ($CAO_2 - CVO_2$) difference (vol %) × coronary blood flow (ml min^{-1} 100 g^{-1} LVW). The oxygen content (CO_2) was determined as follows: $CO_2 = 0.003 \times P_{O_2} + 1.34 \times Hb \times SaO_2$ %/100. Haemoglobin (Hb) concentration (g/100 ml) and the % oxygen saturation (SaO_2) were determined with a hemoximeter (Radiometer, OSM2, Copenhagen, Denmark). Measurements of P_{O_2}, P_{CO_2} and pH in blood samples were made with a blood gas analyser (Radiometer, BMS3-MK2, Copenhagen, Denmark).

Guniea-pigs A similar procedure to that described above was followed for guinea-pigs: the coronary vasculature of guinea-pigs was perfused at a fixed flow rate through the aorta with arterial blood from the right carotid artery of the heparinized donor. The recipient guinea-pigs weighed about 250 g and the donors about 1 kg. Since guinea-pig aortic valves rapidly become incompetent, a polyethylene tube was inserted into the left ventricle by direct wall puncture. Blood in the ventricle was continuously withdrawn and returned to the donor via the venous reservoir.

Aortic perfusion

After anaesthesia with sodium pentobarbitone, recipient rats (about 300 g) were ventilated artificially with room air and thoracotomized by a midline incision. The aorta, the right and left superior and inferior venae cavae were exposed and a polyethylene cannula (i.d. 1.0 mm; o.d. 1.5 mm) was inserted into the aorta via the innominate artery. The descending aorta, and both superior and inferior venae cavae were ligated. A polyethylene tube with a small screw clamp was inserted into the aorta through the LV wall (Figure 1b). The aorta was perfused at a fixed flow rate by pumping the blood from the right carotid artery of a donor rat. A perfusion pressure of about 100 mmHg was maintained by adjusting a screw clamp. The outflow from the aorta was received by a reservoir and in turn returned to the jugular vein of the donor by gravity. The perfusion circuit was the same as that in the coronary perfusion experiments (Figure 1).

Reserpine (Serpasil, Daiichi Seiyaku) (1 mg/kg s.c.) was injected 48 and 24 h before the experiments. This dose of reserpine is sufficient to deplete rat myocardial catecholamine stores by more than 85% (Alpers & Shore, 1969).

Drugs used were acetylcholine chloride (Daiichi Seiyaku), carbachol chloride (Tokyo Kasei), bethanechol chloride (Eisai), tyramine hydrochloride (Daiichi Kagaku), (±)-noradrenaline hydrochloride (Sankyo), phentolamine mesylate (CIBA-Geigy), hexamethonium bromide (Yamanouchi) and atropine sulphate (Takeda). All drugs were dissolved in or diluted with 0.9% w/v NaCl solution (saline) and 0.01 ml of each solution (unless otherwise stated) was injected, over a period of 4 s, into the perfusion system immediately distal to the aortic cannula by means of individual microsyringes (Jintan Terumo Co.). Maximal drug effects were calculated and expressed as % changes from the preadministration control levels.

Values in the text are means ± s.e. mean. Student's *t* test was used for statistical analysis. A *P* value of 0.05 or less was considered statistically significant.

Results

Control observations under resting conditions in spontaneously beating rat hearts

Five heart preparations were used for control observations. The mean perfusion pressure (PP) was set at a value slightly lower than that of the mean systemic blood pressure of the donor rat at the onset of per-

Table 1 Mechanical parameters and myocardial oxygen consumption under resting conditions in spontaneously beating rat hearts

	Arterial		*Venous*
Left ventricular systolic pressure (mmHg)		110 ± 9	
Heart rate (beats/min)		251 ± 17	
Perfusion pressure (mean; mmHg)		102 ± 2	
Coronary inflow (ml/min)		3.2 ± 0.2	
Coronary vascular resistance (10^4 × dyn.s/cm^5)		260 ± 19	
Left ventricular wet weight (g)		0.66 ± 0.02	
Systemic blood pressure of donors (mean; mmHg)		110 ± 3	
P_{O_2}	96 ± 11		61 ± 3
P_{CO_2}	35 ± 1		37 ± 2
Haemoglobin concentration (g/100 ml blood)	14.4 ± 0.3		14.4 ± 0.3
pH	7.394 ± 0.008		7.395 ± 0.008
Oxygen saturation (%)	90 ± 2		74 ± 3
Myocardial oxygen consumption ml/100 g left ventricle			
per min		15.5 ± 0.4	
per beat		0.063 ± 0.005	

Mean ± s.e. mean of 5 observations on 5 preparations. The observations were made 30 to 60 min after the start of perfusion.

fusion; the flow rate was 3.3 ml/min. Shortly after the start of perfusion the pressure rose slightly and reached a new steady-state level; it was then re-adjusted to near 100 mmHg. The peak systolic pressure of the left ventricle increased until it equalled the PP and thereafter remained constant at this level. Most of the preparations had stabilized 20 to 30 min after the beginning of perfusion and thereafter remained virtually stable for about 2 h. At this stage, basal values of mechanical parameters and myocardial oxygen consumption were measured and are shown in Table 1.

Effects of acetylcholine (ACh), carbachol and bethanechol

Rats Single doses of ACh (0.003 to 3 μg), carbachol (0.01 to 1 μg) and bethanechol (0.1 to 30 μg) were injected intra-arterially. ACh in doses of 0.003 to 0.03 μg decreased perfusion pressure (i.e., vasodilatation) without markedly influencing left ventricular pressure (LVP) or heart rate (HR). With further increasing doses, the decreases in perfusion pressure were progressively diminished and finally converted to increases (vasoconstriction). These higher doses decreased LVP and HR in a dose-dependent manner. Carbachol at doses of below 0.1 μg decreased PP, but had almost no effect on LVP or HR. With the dose range between 0.1 to 1 μg, dose-related decreases in LVP and HR and increases in perfusion pressure were observed. In doses below 0.3 μg, bethanechol slightly decreased perfusion pressure, but there were no definite changes in LVP or HR; above 0.3 μg, bethanechol produced dose-dependent decreases in LVP and HR and increases in perfusion pressure. Single injections of adenosine (0.3 to 30 μg) caused only decreases in perfusion pressure, although adenosine produced similar cardiac responses to those caused by the cholinomimetic drugs (Figure 2). Similar responses to these cholinomimetic drugs were also observed in electrically driven hearts (Figure 3) and in preparations in which urethane (1.2 g/kg i.v.) was used as the anaesthetic. Figure 4 presents the dose-response curves for increases in perfusion pressure produced by ACh, carbachol and bethanechol. The injection of equivalent volumes of saline, adjusted to a pH of 6.0, 7.0 or 8.0, and at a temperature equivalent to that of the drug solutions, was without effect on coronary perfusion pressure.

Guinea-pigs Spontaneously beating, donor-perfused guinea-pig hearts stabilized about 20 min after the start of perfusion. Basal values were: perfusion pressure, 85 ± 3 mmHg and coronary flow, 3.9 ± 0.1 ml/min ($n = 6$). Single injections of ACh (0.03 to 3 μg), carbachol (0.01 to 1 μg) and bethanechol (1 to 30 μg) decreased pressure in a dose-dependent manner;

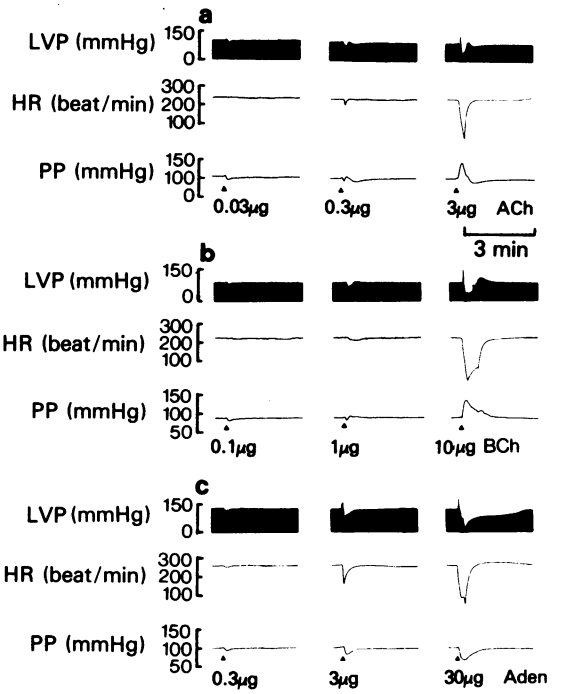

Figure 2 Cardiac and vascular responses to single injections of (a) acetylcholine (ACh), (b) bethanechol (BCh) and (c) adenosine (Aden) into the coronary perfusate of spontaneously beating, donor-perfused rat hearts. LVP, left ventricular pressure; HR, heart rate; PP, mean perfusion pressure.

this decrease was occasionally preceded by a slight increase in pressure, especially immediately after the injection of large doses. The summarized results are presented in Figure 5.

Investigation of the mechanism of vasoconstriction caused by acetylcholine, carbachol and bethanechol in spontaneously beating rat heart preparations

Effects in reserpine-treated preparations The basal values after a 30 min stabilization period were not significantly different ($P > 0.05$) from those of the untreated group (Table 1). When single doses of ACh (1 μg), carbachol (1 μg) or bethanechol (10 μg) were administered into the coronary perfusate, the vascular responses were not significantly modified by reserpine pretreatment (Figure 6a). On the other hand, the increases in LVP (23 ± 3%) and perfusion pressure (15 ± 2%) resulting from the administration of tyramine (30 μg) were greatly reduced by this pretreatment (increases of 7 ± 1% and 4 ± 1% respectively; $P < 0.001$).

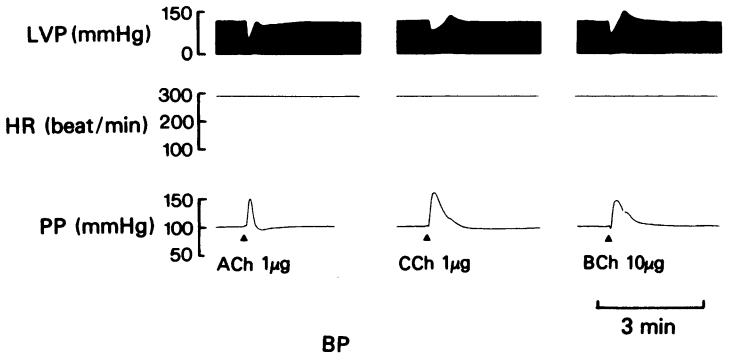

Figure 3 Cardiac and vascular responses to single injections of acetylcholine (ACh), carbachol (CCh) and bethanechol (BCh) into the coronary perfusate of electrically driven, donor-perfused rat hearts. Parameters as in Figure 2.

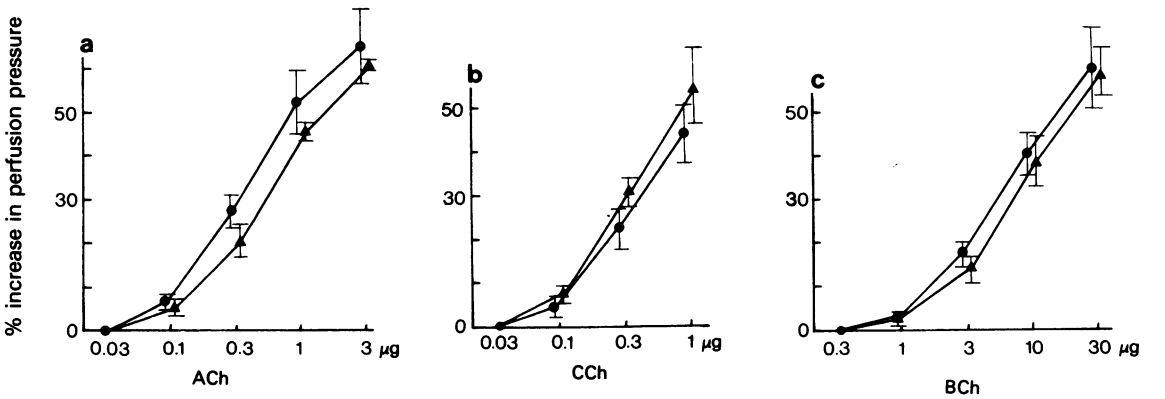

Figure 4 Dose-response curves for changes in mean coronary perfusion pressure induced by (a) acetylcholine (ACh), (b) carbachol (CCh) and (c) bethanechol (BCh). Each point represents the mean of 7 observations on 7 spontaneously beating rat heart preparations (●) and of 5 observations on 5 electrically driven rat hearts (▲). Vertical bars show s.e. mean. There were no significant differences ($P > 0.05$) between the corresponding values from spontaneously beating and electrically driven hearts. Initial perfusion pressure: spontaneously beating hearts, 100 ± 4 mmHg ($n = 7$); electrically driven hearts, 101 ± 3 mmHg ($n = 5$); $P > 0.05$.

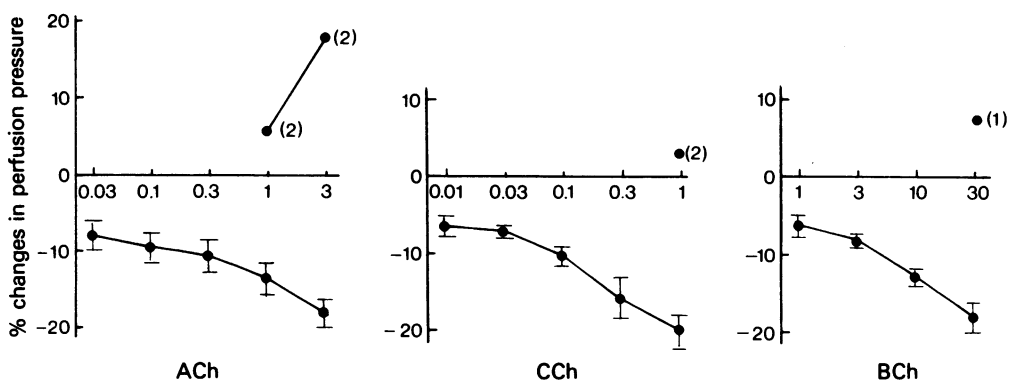

Figure 5 Vascular responses to single injections of acetylcholine (ACh), carbachol (CCh) and bethanechol (BCh) into the coronary perfusate of spontaneously beating, donor-perfused guinea-pig hearts. Each point represents the mean of 6 observations on 6 preparations. Vertical bars show s.e. mean. The frequency of occurrence of an initial increase in the perfusion pressure is shown in parentheses.

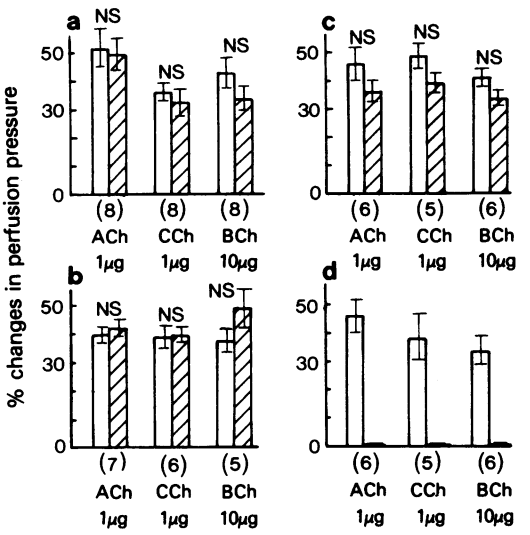

Figure 6 Influence of various blocking agents on the vasoconstrictor responses to acetylcholine (ACh), carbachol (CCh) and bethanechol (BCh) in spontaneously beating, donor-perfused rat hearts. Open columns, untreated; hatched columns, treated. Vertical bars represent s.e. mean and the number of experiments is given in parentheses. NS, not significant. (a) Reserpine pretreatment (subcutaneously in a dose of 1 mg/kg 48 and 24 h, before the experiment); (b) phentolamine (100 to 200 μg i.a.); (c) hexamethonium (1 mg i.a.); (d) atropine (3 μg i.a.).

Influence of phentolamine Single injections of phentolamine (100 to 200 μg) into the coronary perfusion circuit transiently (i.e. for 30 to 60 s) and slightly decreased LVP, HR and PP. These doses of phentolamine significantly reduced the increases in PP (before, 15 ± 1%; after 3 ± 1%; $P < 0.001$; $n = 7$) and LVP (before, 36 ± 6%; after, 11 ± 4%; $P < 0.02$; $n = 5$) caused by single injections of noradrenaline (0.1 μg). This antagonism by phentolamine lasted for over 30 min. In contrast, the vasoconstriction induced by local administration of ACh (1 μg), carbachol (1 μg) and bethanechol (10 μg) was not modified (Figure 6b). The doses of phentolamine used were sufficient, in intact rats, to convert a pressor response to adrenaline (3 μg/kg i.v.) to a depressor one.

Effect of hexamethonium A single dose of hexamethonium (1 mg) was given intra-arterially a few minutes before examining the effects of ACh (1 μg), carbachol (1 μg) or bethanechol (10 μg). This dose of hexamethonium, which itself induced slight positive inotropic and chronotropic responses and vasodilatation, failed to block the vasoconstrictor responses to the cholinomimetic drugs (Figure 6c).

Effect of atropine A single intra-arterial injection of atropine (3 μg) had little effect on the measured cardiac parameters. This dose of atropine antagonized both the vasoconstrictor and vasodilator responses of ACh (1 μg), carbachol (1 μg) and bethanechol (10 μg) (Figure 6d). This antagonism lasted for 15 to 30 min.

Effects of acetylcholine, carbachol and bethanechol on aortic perfusion

In three preparations the cannulated aorta was perfused at a fixed flow rate of 2.8 ± 0.1 ml/min, i.e., equivalent to the flow rate used in the coronary perfusion studies. The mean perfusion pressure was maintained at 92 ± 2 mmHg by adjusting a small screw clamp (see Figure 1b). The preparations stabilized about 10 min after the start of perfusion. Single doses of ACh (1 to 3 μg), carbachol (1 to 3 μg) and bethanechol (10 to 30 μg), sufficient to induce a definite rise in coronary perfusion pressure, were injected into the aortic perfusion circuit. These cholinomimetic drugs had almost no effect on aortic perfusion pressure.

Discussion

In the present experiments single injections of large doses of ACh, carbachol and bethanechol into the coronary perfusate of donor-perfused rat hearts increased the perfusion pressure both in spontaneously beating and electrically driven hearts. The increase in perfusion pressure was accompanied by almost no ejection of blood from the left ventricle, probably indicating that the aortic valves were competent. This was confirmed by inserting a needle through the left ventricular wall into the ventricle cavity. Since the arterial blood was conducted into the coronary vasculature at a fixed flow rate, changes in the perfusion pressure indicate vascular responses; an increase, vasoconstriction and a decrease, vasodilatation. Although alterations in extravascular myocardial compression also change coronary vascular resistance (Rushmer, 1961) it should be noted that, in the present experiments, the increase in coronary artery perfusion pressure induced by the cholinomimetic drugs was accompanied by negative, rather than positive, inotropic and chronotropic responses.

In the present experiments, the cholinomimetic drugs were injected into the coronary perfusion circuit via the aortic cannula. Since Furchgott (1954) found that ACh contracts aortic strips, the question arises as to whether drug-induced changes resulted from effects on aortic, rather than coronary vascular smooth muscle. This possibility can be ruled out, because these cholinomimetic drugs were without effect on the perfused aortic preparation.

Hashimoto *et al.* (1960), who controlled perfusion pressure, heart rate and extravascular compression by using fibrillating dog hearts with an isolated perfused coronary circulation, showed a substantial increase in coronary blood inflow after intra-coronary injections of acetylcholine. Similar findings have been reported by the other workers using dog hearts (Katz *et al.*, 1938; Shreiner *et al.*, 1957; Blumental *et al.*, 1968; Levy & Zieske, 1969; Sakai *et al.*, 1974). Thus, there is general agreement that ACh, when administered into the coronary perfusion, is a potent vasodilator. The present results also demonstrate that in low doses all those cholinomimetic drugs were dilator; it was only in much larger doses that a constrictor effect was observed.

One possible explanation for this vasoconstriction is an indirect effect, via released noradrenaline, on α-adrenoceptors present in coronary vascular smooth muscle (Berne, 1964; Ross, 1976). ACh has certainly been shown to release myocardial catecholamines in various animal species (Higgins *et al.*, 1973; Westfall, 1977). There was no evidence of this in the present experiments, since the acetylcholine-induced vasoconstriction was not affected by pretreatment with reserpine and by acute administration of phentolamine or hexamethonium. In contrast, the vascular, as well as the cardiac, responses induced by these cholinomimetic drugs were extremely susceptible to the blocking action of small doses of atropine. These facts indicate that the vasoconstrictor responses involved a direct action on muscarinic receptors located in the coronary vasculature. ACh vasoconstriction (which could be antagonised by atropine) has also been demonstrated using isolated large coronary arteries obtained from pigs (Takenaka, 1959; Bayer, Mentz & Förster, 1974) and rabbits (Norton, Gellai & Detar, 1972; De La Lande, Harvey & Holt, 1974), but not from dogs (Toda, 1974; Toda, Hojo, Sakae & Usui, 1975) or kittens (Cornish, Miller & Tolmer, 1974).

The present results suggest two distinct types of muscarinic receptor sites in the coronary vasculature of the rat heart: type I sites respond to small doses of the cholinomimetic drugs and result in vasodilatation; type II sites respond to large doses and result in vasoconstriction.

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