MOTOR INNERVATION OF THE CORONARY ARTERIES OF THE CAT

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SUMMARY

1. The effect on coronary vascular resistance of selective stimulation of the Aδ, B and sC fibre groups of the post-ganglionic cardiac sympathetic nerves was studied. The main left coronary artery was perfused at constant flow. The oxygen saturation of coronary sinus blood was measured continuously.

2. Stimulation of the peripheral ends of the cut Aδ afferent fibres, normally excited by myocardial ischaemia, had no effect on coronary vascular resistance; these fibres do not evoke an axon reflex in the heart.

3. Stimulation of the preganglionic B fibres that run without synapse through the stellate ganglion also had no measurable effect on coronary resistance.

4. Stimulation of the post-ganglionic sC fibres of the cardiac sympathetic nerves caused coronary vasodilatation which occurred earlier than, and was initially independent of the decrease in coronary sinus oxygen saturation.

5. The injection of noradrenaline into the perfusion system had the same effect as stimulation of the sC fibres. In the K^+-arrested heart, both noradrenaline and stimulation of the post-ganglionic nerves elicited coronary vasodilatation without changing the oxygen saturation of coronary sinus blood.

6. The intracoronary injection of acetylcholine caused coronary vasodilatation followed by an increase of coronary sinus oxygen saturation.

7. Vagal stimulation caused bradycardia and a fall in coronary resistance.

8. Propranolol blocked coronary vasodilatation elicited by sympathetic stimulation or noradrenaline without affecting the vasodilatation due to myocardial ischaemia or acetylcholine. Atropine blocked coronary vasodilatation evoked by acetylcholine without affecting that due to ischaemia or noradrenaline. Therefore smooth muscle of the coronary arteries has at least three different receptor sites from which vasodilatation can be elicited.

9. Hypertensin caused coronary vasoconstriction.

10. The presence of sympathetic cholinergic vasodilator fibres innervating the coronary arteries could not be demonstrated.

INTRODUCTION

These experiments were undertaken to determine the effect on coronary vascular resistance induced by selective stimulation of the Aδ, B and sC fibre groups known to be present in the cardiac sympathetic nerves (Brown, 1967a). The afferent Aδ fibres can be excited by myocardial ischaemia and can initiate a pseudaffective reflex (Brown, 1967a); it was therefore of interest to look for the presence of an axon reflex in the coronary arteries similar to the one that occurs during noxious stimulation of the conjunctiva (Bruce, 1913). Both coronary vasodilatation and vasoconstriction have been ascribed to the influence exerted by preganglionic group B fibres that run without synapse through the stellate ganglion in the cardiac sympathetic nerves (Kiss & Szentiványi, 1957; Juhász-Nagy & Szentiványi, 1961). Such results were not confirmed by others (Gregg & Fisher, 1963), but the electrical activity of the stimulated nerves was not monitored in either instance. Finally, there is disagreement regarding the effect on coronary vascular resistance evoked either by stimulation of postganglionic sC sympathetic fibres or by intracoronary injections of noradrenaline. It is generally agreed that the major effect is coronary vasodilatation; however, the latter has been attributed to increased myocardial metabolism elicited by cardiac sympathetic stimulation (Berne, 1964). When myocardial metabolism is limited by arresting the heart of the dog, noradrenaline may cause vasoconstriction (Berne, 1958). On the other hand, Gaal, Kattus, Kolin & Ross (1966) always found vasodilatation using noradrenaline, a vasoconstrictor effect being elicited only after pretreatment with propranolol, a β-adrenergic blocker. In the present experiments, the relation between coronary vasodilatation and increased myocardial metabolism elicited by stimulation of the cardiac sympathetics or noradrenaline was studied by simultaneously recording an index of coronary resistance and coronary sinus oxygen saturation. The experiments were done in cats because the grouping of cardiac sympathetic nerve fibres was known for this species; moreover, techniques for perfusing the coronary arteries have been established (Brown, 1965). The effects of intracoronary injection of acetylcholine and of vagal stimulation were studied also.

METHODS

Forty-four cats weighing 2–5 kg were anaesthetized with intraperitoneal injection of pentobarbitone 40 mg/kg (Diamond Laboratories). The animals were paralysed using gallamine triethiodide (Flaxedil; Davis and Geck) 2 mg/kg injected intravenously; supplemental doses (1 mg/kg) were given as required. Periodically these animals were allowed to recover from paralysis to ensure that pinching the hind limb did not provoke a pain-like reaction; if this occurred supplemental doses of anaesthetic ($\frac{1}{4}$–$\frac{1}{5}$ the initial dose) were given. Positive pressure respiration was supplied by a Harvard respiration pump. The stroke and rate were adjusted to maintain the end expiratory CO_2 at 35 mm Hg measured with an infra-red CO_2 analyser (Beckman). The arterial pH measured by a Beckman pH meter varied between 7·37 and 7·42 and arterial oxygen saturation measured by the Van Slyke method varied from 90 to 95 %. Rectal temperature was maintained at 37° C by a thermostatically controlled heating unit attached to the animal board. A polythene catheter was inserted into one common carotid artery and passed retrograde to the aortic arch. The catheter was connected to a strain gauge (Statham P 23 dB) for measurement of aortic blood pressure. The dynamic characteristics of the catheter-manometer systems have been described (Brown, 1967a). The pressure pulse signal triggered a frequency meter (Hewlett Packard 500 BR) which registered the heart rate. Both cervical vagus nerves were sectioned, the right stellate ganglion crushed and the left stellate and its branches exposed and disconnected centrally according to the method previously described (Brown, 1967a). Heparin (liquemin sodium, Organon) was injected intravenously (500 u./kg) and an additional 500 u. was given every 30 min for the duration of the experiment which usually lasted 2–4 hr. At the end of each experiment the heart was removed, washed free of blood and blotted on cotton gauze; the left ventricle and the interventricular septum were weighed. The average weight was 12 ± 2 g.

Coronary artery perfusion. The main left coronary artery was exposed, cannulated and perfused in a manner similar to that previously described (Brown, 1965) (Fig. 1). The major modification was that the flow was kept constant over a pressure range of 0–300 mm Hg by a constant flow peristaltic pump (Harvard Co. Dover, Mass.). Pressure was related to the resistance of the left coronary vascular bed by the equation $\Delta P = R \times F$, where ΔP is coronary arterial minus coronary sinus pressure, R is coronary resistance and F, coronary inflow. Changes in coronary arterial pressure approximately equalled changes in coronary resistance since inflow was constant and coronary sinus pressure changed relatively slightly and usually not at all. The two were directly related when coronary sinus pressure was unchanged. Pressure was measured with a Statham strain gauge in the inflow circuit at the site indicated (by T) in Fig. 1. The signal was passed through a passive RC network with a time constant of 2·0 sec to obtain mean pressure values. This was one-eighth to one-tenth the time to the maximum arterial pressure effect evoked by the different procedures used in these experiments. The periodic variation of inflow pressure was related to the frequency of the pump. The volume of the inflow circuit was 15 ml. and it was primed with Locke solution of the following composition: NaCl, 126 mM; KCl, 5·64 mM; $CaCl_2$, 2·89 mM; Tris buffer (Sigma 121), 6·0, g/l. The temperature of the blood was monitored continuously with a thermistor probe (Fig. 1) and maintained at 37° C by a thermostatically controlled water jacket. The perfusion tubing was polythene; it was coated with silicone (Siliclad; ClayAdams Inc.).

The coronary flow varied between 15 and 20 ml./min or 1·0 and 1·5 ml./g left ventricle.min compared to a flow of 0·8 ml./g.min measured with a bubble flowmeter in the naturally perfused main left coronary artery of the cat (Brown, 1964). With the flows used the pressure measured in the arterial perfusion circuit was generally 10–30 mm Hg greater than that in the aorta. In six experiments, the flow was varied over a range of 0·5–2·0 ml./g.min and the pressure over a range of 30 mm Hg to less than 50 mm Hg greater than the aortic

pressure, without affecting the results. Towards the end of an experiment the coronary resistance gradually rose and the aortic pressure fell. This may have been due to haemolysis caused by the pumps since the plasma haemoglobin rose from 2·0 to 8·0 mg/100 ml. When this occurred, the experiments were terminated.

Measurement of the oxygen saturation of coronary sinus blood. A stainless-steel cannula (1·5–2·0 mm o.d.) was inserted into the right atrium and passed into the coronary sinus at its junction with the inferior vena cava where it was tied into place. The coronary sinus
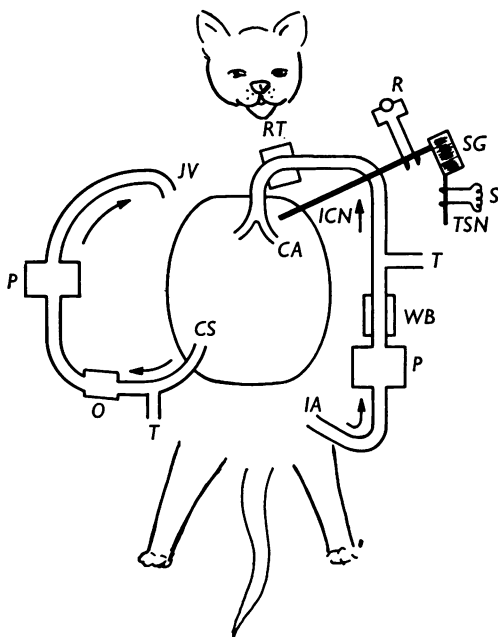

Fig. 1. Diagram of the experimental arrangement. *T*, strain gauge; *P*, constant flow peristaltic pumps; *O*, flow-through cuvette oximeter; *RT*, rubber tubing through which drugs were injected and in which a thermistor was placed; *WB*, thermostatically controlled water-bath; *CA*, main left coronary artery; *CS*, coronary sinus; *JV*, external jugular vein; *IA*, common iliac artery; *SG*, stellate ganglion; *TSN*, preganglionic thoracic sympathetic nerve trunk; *ICN*, inferior cardiac nerve; *S*, stimulating electrodes; *R*, recording electrodes. Arrows indicate direction of blood flow.

blood was withdrawn at a constant rate of flow through a cuvette oximeter (Waters Conley, Rochester, Minn.) using a second peristaltic pump. The blood was returned to the animal via a catheter which had been inserted into an external jugular vein and passed forward to the superior vena cava (Fig. 1). Mean pressure in this circuit was measured by a strain gauge. The volume between the tip of the cannula and the outlet of the oximeter was 1·5 ml. The output of the oximeter was calibrated against the haemoglobin O_2 saturation (S_{a,O_2}), determined by the method of Van Slyke, and gave the curve shown in Fig. 2*A*. It was neither linear nor logarithmic over the entire range but over the range of most of the changes encountered in the present experiments (between 40 and 60 % S_{a,O_2}) the relationship was approximately linear. There was negligible DC drift during an experiment. The oximeter had a time constant of 10 msec and there was no distortion of the saturation profile as the

blood was pumped through the cuvette because a square wave input at the tip of the cannula elicited a square wave response from the oximeter. The square wave changes were produced by withdrawing samples of blood having different S_{a,O_2} through the coronary sinus perfusion circuit (Fig. 2B). The lag time between the tip of the cannula and the beginning of the oximeter response was determined in this way in twenty-three experiments; it varied from 6 to 10 sec depending upon the flow rate. Identical values were calculated from the volume/flow relation of this part of the perfusion circuit. Such values were 0·5–1·0 sec less than those obtained in twenty-three experiments by injecting a slug of Indocyanine Green dye (Hynson, Westcott and Dunning, Inc.) into the rubber tubing of the perfusion circuit (Fig. 1) indicating that the circulation time of the fastest dye particles (appearance time) through the coronary bed was 0·5–1·0 sec. The longest appearance time (1·0 sec) was added to the lag time giving the total lag time, which is a measurement of the appearance time of coronary arterial blood at the cuvette; the total lag time therefore slightly over-estimates the appearance time of coronary capillary blood.

The saturations of coronary sinus blood in these experiments fell on the nearly linear part of the oxyhaemoglobin dissociation curve for cat blood (Dittmar, 1961) and thus indicate proportionate changes in oxygen tension. The oxygen tension of the coronary sinus blood in turn reflects the oxygen tension in the capillary bed and myocardium. It was therefore assumed that the changes in coronary sinus oxygen saturation after correction for total lag time represented accurately changes in myocardial oxygen tension and support for this view came from experiments in which the coronary inflow was stopped for brief periods (Fig. 3) or when venous blood was injected into the arterial inflow line.

The coronary sinus flow was adjusted to maintain the coronary sinus pressure between 0 and 10 mm Hg and was usually $\frac{2}{3}$–$\frac{3}{4}$ the inflow. The entire outflow came from the inflow since it ceased when the inflow was stopped and was unaffected by occlusion of the right coronary artery. The right coronary artery of the cat was $\frac{1}{4}$–$\frac{1}{5}$ the size of the left.

Measurement of coronary blood volume. In ten experiments, the indicator–dilution curves produced by the injection of Indocyanine Green were analysed for the mean transit time of the dye particles through the coronary circulation using the equation

$$\text{Mean transit time} = \frac{\Sigma c(t)}{\Sigma c},$$

where c is the concentration of the dye at each 0·25 sec (t) (Zierler, 1958). The curves were recorded on tape and the mean transit times were determined using a 3200 CDC (Control Data Corp.) digital computer which was preprogrammed with the foregoing equation. Extrapolation to eliminate the effects of recirculation was accomplished by finding the minimum of $d^2(\log c(t))/dt^2$ and fitting an exponential slope to this minimum; the programme was described by Stauffer, Pryor, Gardner, Day & Warner (1966).

Coronary blood volume was determined from the product of coronary sinus flow and mean transit time and, since the flow was constant, the mean transit time was an index of the coronary blood volume. When this method was applied to known volumes, the error did not exceed 15–20 %.

Stimulation of cardiac sympathetic and vagus nerves. The method of stimulating the preganglionic thoracic sympathetic trunk and recording from the intact post-ganglionic cardiac sympathetic nerves has already been described (Brown, 1967b). The Aδ elevation was evoked by voltages (0·8 V; 0·08 msec) which are below threshold for the preganglionic B fibres. In order to stimulate selectively those B fibres that ran without synapse through the stellate, the post-ganglionic nerves were stimulated at voltages (1·2 V; 0·08 msec) just threshold for the B elevation recorded antidromically in the preganglionic nerve; at these voltages there was no sC elevation in the post-ganglionic nerves. The latter was usually elicited by supramaximal stimulation of the preganglionic nerve (10 V; 0·08 msec); occasionally the sC fibres of the post-ganglionic nerves were stimulated directly (30 V; 0·3 msec). The peripheral end

of the cut left vagus was stimulated in the neck, the cervical sympathetic having been sectioned caudal to this level. Stimulus parameters which elicited a maximal bradycardia were used (10 V, 5 msec, 20/sec). The trains of stimuli used for sympathetic and vagal stimulation were both generally 10 sec in duration.

Drug injections. Drugs dissolved in Locke solution were injected directly into the coronary arterial perfusion circuit near the coronary cannula (Fig. 1). The volume between injection site and tip of coronary cannula was 1·0 ml. A precision-drilled microlitre syringe (Hamilton
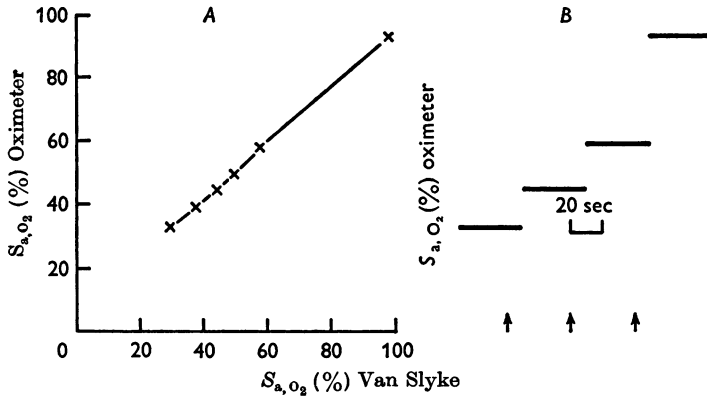

Fig. 2A. Output of the cuvette oximeter (ordinate) plotted against the results obtained by the method of Van Slyke (abscissa) using identical blood samples. S_{a,O_2} = oxygen saturation. For values between 40–60 % the relationship was approximately linear (*B*). Response of the cuvette oximeter to blood samples having different S_{a,O_2} values; the changes were made at the arrows. The interval between each arrow and the subsequent step increase of S_{a,O_2} is due to the dead space between the tip of the cannula and the distal end of the oximeter and was referred to as the lag time.

Co., Whittier, California) was used. The volume injected was always less than 10 μl. because larger volumes of Locke solution alone caused slight coronary vasodilatation. Similar volumes were used for the same reason by Gaal *et al.* (1966). The following drugs were used: noradrenaline (Winthrop), acetylcholine (Merck and Co.), hypertensin (Ciba), propranolol (Ayerst) and dibenzylene (Smith, Kline and French).

In ten experiments, pulmonary arterial flow was measured by an electromagnetic flow probe placed around the main pulmonary artery. The method of calibrating this probe has been described (Anderson & Brown, 1967).

The results were recorded on a Honeywell Visicorder (Model 1508) and a 7-channel tape recorder (Ampex FR 1300).

RESULTS

Effect of reduced coronary inflow. Figure 3 shows the effects of stopping coronary inflow for periods of 5·0 and 2·5 sec. The pressure in the inflow circuit fell abruptly but did not reach zero because there was a run-off of blood still present in the tubing between the pump and the coronary cannula. When flow was resumed a gradual recovery (half-time, 6 sec) of coronary arterial pressure occurred. Changes of coronary sinus pressure

were parallel to but much smaller than changes of inflow pressure. There-
fore calculated coronary vascular resistance also fell with a similar time
course. Since the heart rate, aortic blood pressure, and cardiac output
were unchanged during such brief arrests of inflow, it was expected that
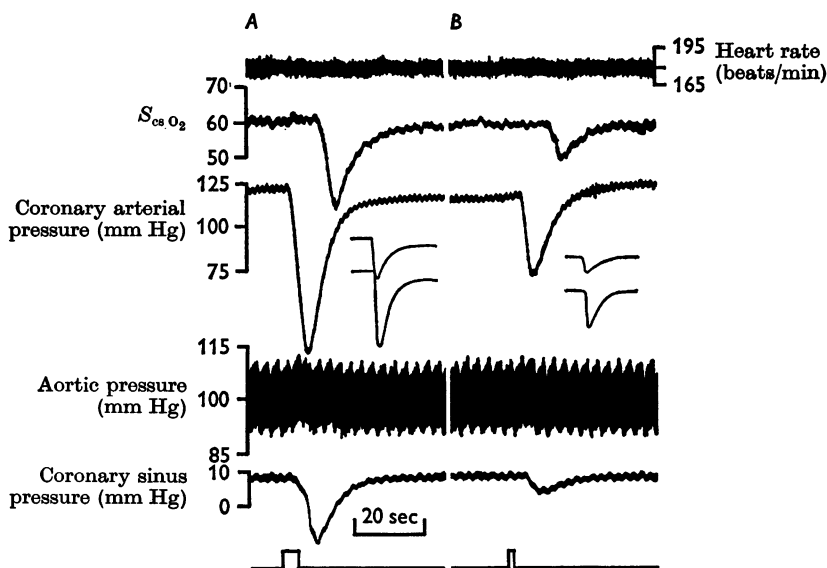coronary sinus oxygen saturation also would be sharply reduced as was
the case.

Fig. 3. Effects of stopping the coronary arterial inflow at the signals, for 5 sec
(*A*) and 2·5 sec (*B*). From top to bottom, heart rate (beats/min); S_{cs,O_2} = coronary
sinus oxygen saturation (%); coronary arterial pressure (mm Hg); aortic pressure
(mm Hg); coronary sinus pressure (mm Hg). Bottom trace was used both for mark-
ing events and as a reference line for all parameters. The insets of (*A*) and (*B*)
show the relation between coronary sinus oxygen saturation and coronary arterial
pressure after correcting for the 10 sec total lag time of the coronary sinus
oxygen saturation trace. Note the striking parallel between the recovery curves
for coronary sinus oxygen saturation and coronary arterial pressure; also note
the initial rapid, steep fall in coronary sinus oxygen saturation.

The half-time for recovery of coronary sinus oxygen saturation was
5 sec. Moving the coronary sinus oxygen saturation curve 10 sec ahead
in time (Fig. 3, inset), to correct for the total lag time, shows how closely
related the changes in coronary arterial pressure were to changes in
coronary sinus oxygen saturation. Longer periods of arrested inflow caused
greater falls of both coronary arterial pressure and coronary sinus oxygen
saturation (Fig. 3); the half-times of recovery for both were also pro-
longed. Figure 4 shows a plot of the half-time of recovery of coronary
arterial pressure versus half-time of recovery of coronary sinus oxygen

saturation for ten different periods of arrested inflow varying from 1 to 5 sec in duration. There was a high degree of correlation ($r = 0.84$ and $P < 0.005$).

Therefore, during recovery, changes in coronary resistance were probably due to changes in myocardial oxygen tension as reflected in the coronary sinus oxygen saturation trace. This agrees with the well-known finding of Hilton & Eicholtz (1925), that myocardial hypoxia is a potent coronary vasodilator. It is unlikely that an accumulation of CO_2 was involved since hypercapnia is not a strong coronary vasodilator (Berne, 1964). The
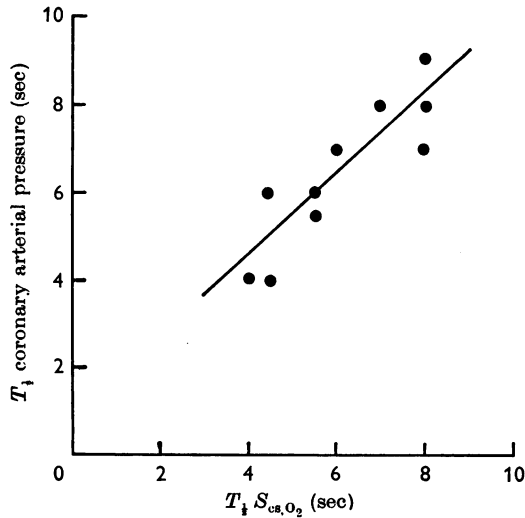

Fig. 4. Half-time ($T_{\frac{1}{2}}$) of recovery of coronary arterial pressure plotted against half-time for recovery of coronary sinus oxygen saturation (S_{cs,O_2}) for 10 experiments in which coronary inflow was stopped for periods from 1–5 sec. Calculated regression line is drawn through the points. The correlation was highly significant, $r = 0.84$, $P < 0.005$.

possibility that changes in vascular myogenic tone resulting from the abrupt fall in coronary pressure may have been implicated was assessed by using a variety of different flow rates. Changes in inflow were always accompanied by abrupt changes in pressure which remained steady for at least 5–10 min. It appears that in these experiments, the influence of pressure-dependent changes in vascular myogenic tone was negligible.

Effect of stimulation of $A\delta$ fibres. In five experiments, stimulation of the peripheral end of the cut thoracic sympathetic preganglionic nerve at 5–150/sec with voltages above threshold for the $A\delta$ fibres but below that of preganglionic B fibres, had no effect on coronary arterial pressure, coronary sinus oxygen saturation, aortic blood pressure, pulmonary arterial flow, heart rate, right atrial or coronary sinus pressures.

Effect of stimulation of preganglionic B fibres that ran without synapse through the stellate ganglion. Stimulation of the cardiac sympathetic nerves distal to the stellate ganglion at 1–80/sec and voltages just threshold for B fibres provoked a very small rise (5 mm Hg) in aortic blood pressure without any change in the other parameters in two out of five experiments; in the remaining three experiments there was no measurable effect.
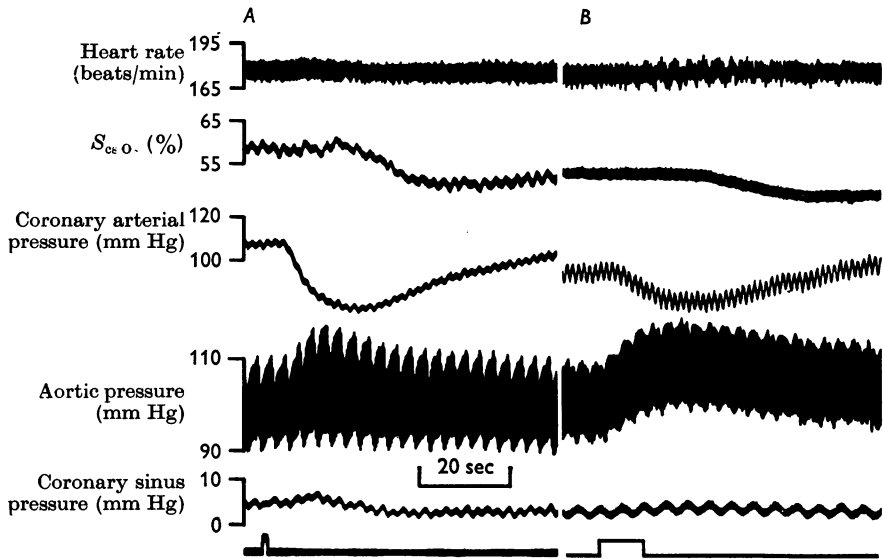

Fig. 5. Effect of injecting noradrenaline (0·05 μg) directly into the perfusion circuit at the signal (A) and of stimulating supramaximally the preganglionic thoracic sympathetic nerve at 20/sec for 10 sec at the signal in panel B. Notation as in Fig. 3. After correcting for the 10 sec lag time of coronary sinus oxygen saturation, the fall in coronary arterial pressure began earlier and was much faster. During recovery however these two parameters ran a more parallel course.

Effect of stimulating the post-ganglionic sC fibres in the cardiac sympathetic nerves and of the intracoronary injection of noradrenaline. Since noradrenaline is the transmitter released from sympathetic endings in the heart (Euler, 1959) it was expected that similar effects would result using either nerve stimulation or intracoronary injection of noradrenaline, and such was the case. Figure 5 shows the results obtained using either procedure. There was always a fall in coronary sinus oxygen saturation and coronary arterial pressure, a rise in both aortic and coronary sinus pressures and an increase in cardiac output (not shown here). Therefore the fall in coronary resistance was proportionately greater than the fall of coronary arterial pressure. The heart rate was unchanged probably because the control level was so high (185 $\pm$ 5 beats/min). At the nadir of coronary arterial pressure, the systolic ejection time measured from the aortic

pressure pulse was unchanged from control (25 ± 1 sec/min) (nine experiments). The fall in coronary arterial pressure began before the fall in coronary sinus oxygen saturation (after correcting for total lag time) and reached its nadir earlier. Similarly the half-times for both the fall and recovery of coronary arterial pressure were significantly shorter than the half-times for coronary sinus oxygen saturation. The differences of latencies and half times between the changes in coronary arterial pressure and

TABLE 1. Comparison of the latencies and half-times between the changes in coronary arterial pressure and coronary sinus oxygen saturation (after correction for total lag time) elicited by stimulation of cardiac sympathetics, noradrenaline and acetylcholine

	Latency to onset (sec)			Latency to peak (sec)		
	Coronary arterial pressure		Coronary sinus oxygen saturation	Coronary arterial pressure		Coronary sinus oxygen saturation
Sympathetic stimulation	6.0 ± 0.9	$P < 0.01$	10.3 ± 0.9	20.0 ± 0.7	$P < 0.001$	40.0 ± 2.6
Noradrenaline	4.7 ± 0.4	$P < 0.001$	10.4 ± 1.5	20.2 ± 1.7	$P < 0.001$	33.6 ± 1.5
Acetylcholine	4.7 ± 0.3	$P < 0.001$	10.6 ± 1.7	28.7 ± 1.3	$P < 0.001$	34.8 ± 1.7

	Half-time to greatest effect (sec)			Half-time to recovery (sec)		
	Coronary arterial pressure		Coronary sinus oxygen saturation	Coronary arterial pressure		Coronary sinus oxygen saturation
Sympathetic stimulation	7.5 ± 1.3	$0.025 < P < 0.05$	10.8 ± 0.3	16.3 ± 2.4	$P < 0.001$	48.3 ± 5.9
Noradrenaline	3.6 ± 0.2	$P < 0.001$	12.6 ± 0.7	20.4 ± 0.9	$P < 0.001$	38.6 ± 1.2
Acetylcholine	6.8 ± 0.9	$0.10 < P < 0.20$	5.8 ± 0.7	30.3 ± 1.2	$P < 0.001$	22.8 ± 2.6

Results are mean values ± 1 S.E.M.

coronary sinus oxygen saturation for five experiments in which the preganglionic trunk was supramaximally stimulated at frequencies of 20/sec and for six experiments in which noradrenaline (0·05 μg) was injected into the coronary artery are shown in Table 1.

One factor which could contribute to the differences in latencies and half-times between the changes in coronary arterial pressure and coronary sinus oxygen saturation is a change in the capacity of the coronary vascular bed. In ten experiments, the coronary blood volume ranged from 4 to 6 ml./ 100 g left ventricle, which is lower than the value of 6 to 8 ml./100 g reported by Gregg & Fisher (1963). Neither coronary blood volume nor appearance time of the injected dye particles was measurably altered during sympathetic stimulation or following noradrenaline. However, a

steady state was not attained during either of these procedures which makes measurement of appearance and mean transit times unreliable (Zierler, 1958).

A graph of the half-time of coronary arterial pressure plotted against the half-time of coronary sinus oxygen saturation for six different doses of noradrenaline ranging from 0·01 to 0·10 μg is shown in Fig. 6. There was no correlation between the half-times during the early part of the response when both coronary arterial pressure and coronary sinus oxygen saturation were falling ($r = 0·08$) but a high degree of correlation was present during the recovery ($r = 0·93$, $P < 0·01$).
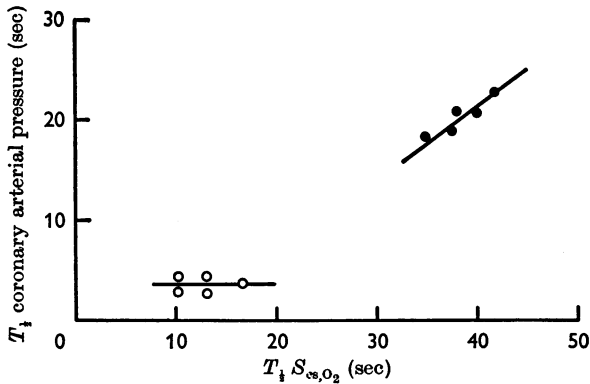

Fig. 6. Graph of the half-time of fall of coronary arterial pressure against half-time of fall of coronary sinus oxygen saturation (open circles), and half-times of recovery (filled circles). Six different doses of noradrenaline ranging from 0·01 to 0·10 μg were injected. Calculated regression lines are drawn through the points. There was no correlation between the early half-times when coronary arterial pressure and coronary sinus oxygen saturation were both falling, $r = 0·08$, but a significant degree of correlation was present during recovery, $r = 0·93$, $P < 0·01$.

When the tape recordings were played back at faster speeds, it was clear that noradrenaline always elicited a fall in coronary arterial pressure before there was any change in aortic blood pressure. However, with nerve stimulation the rise in aortic pressure preceded or occurred at the same time as the fall in coronary arterial pressure. With either method the fall in coronary arterial pressure continued while the aortic pressure was rising.

It was often possible to adjust the dose of noradrenaline so that its effect on aortic blood pressure was small while its effect on coronary arterial pressure remained substantial. Stimulation of the preganglionic trunk had smaller effects at lower frequencies. It was never possible to obtain one parameter of the measured response without the others by varying the frequency. Direct stimulation of the post-ganglionic sC fibres always pro-

duced the same response as supramaximal stimulation of the pre-ganglionic trunk.

In five experiments, 30 ml. arterial blood was withdrawn into a reservoir connected to the coronary inflow line. The heart was arrested by adding 1·5–2·5 mM-KCl to the blood in the reservoir. Note (Fig. 7) that coronary sinus oxygen saturation was greatly increased in the K$^+$-arrested heart. Intracoronary injection of noradrenaline (1 μg) caused a decrease in coronary arterial pressure without change in coronary sinus oxygen saturation in three experiments (Fig. 7A). Supramaximal stimulation of the post-ganglionic sympathetic nerves (two experiments) also caused a fall in coronary arterial pressure without a change in coronary sinus oxygen saturation (Fig. 7B).
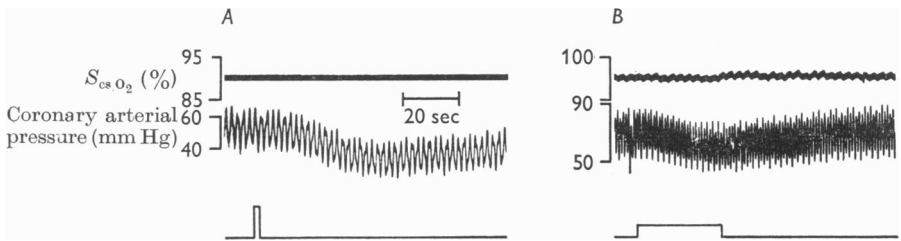

Fig. 7. Effect of noradrenaline (1 μg) (A) and supramaximal stimulation at 10/sec of the post-ganglionic sympathetic nerve (B) on coronary sinus oxygen saturation (top) and coronary arterial pressure (middle) after arresting the heart by adding 1·5–2·5 mM-KCl to the reservoir of arterial blood. The larger fluctuations of the coronary arterial pressure in this figure were due to a shorter time constant of the RC network used to obtain mean pressure.

The possibility that the vasodilatation elicited by stimulation of the cardiac sympathetics was due to sympathetic cholinergic fibres was tested in three experiments. Atropine in doses of 1·0 mg/kg injected intravenously blocked coronary vasodilatation evoked by intracoronary injection of acetylcholine (ACh) or vagal stimulation but had no effect on the response evoked by sympathetic stimulation, or stopping the inflow.

Propranolol in doses of 1·0 mg/kg injected intravenously blocked the response to sympathetic stimulation and noradrenaline; no rise in coronary arterial pressure was observed (ten experiments). This drug had no effect on the fall in coronary arterial pressure elicited either by stopping inflow or by ACh.

Dibenzylene, an α-adrenergic blocker was injected intravenously (1·0 mg/kg) in three experiments; in two of these, propranolol, a β-adrenergic blocker, had been given previously. The response to sympathetic stimulation did not appear to be altered by this drug in either case.

In three of the experiments in which the preganglionic trunk was stimu-

lated, a slight rise in coronary arterial pressure preceded the large fall. This occurred when the increase of aortic pressure was greater than usual. In two of these experiments, propranolol abolished both the early small rise and the late large fall of coronary arterial pressure. Therefore, the small, early increase was probably due to the increase of intramyocardial tension that must have occurred at the same time. Such increases of coronary arterial pressure were never seen after noradrenaline.

Fig. 8(*A*). Effect of the injection of acetylcholine (0·05 μg) directly into the inflow at the signal. Notation as in Figs. 3, 5. Note the rapid, abrupt fall in coronary arterial pressure and the small rise in coronary sinus oxygen saturation secondary to the small fall in aortic pressure. (*B*) Effect of stimulation of the left cervical vagus.

Effect of the intracoronary injection of acetylcholine. In ten experiments, ACh was injected into the coronary artery in doses ranging from 0·01 to 0·1 μg. In each case there was an initial fall in coronary arterial pressure despite an increase of coronary sinus pressure (Fig. 8*A*); next a fall in aortic blood pressure followed in turn by an increase of coronary sinus oxygen saturation occurred. The heart rate was unchanged. Table 1 shows that there was a significant difference in the latencies to onset and peak between coronary arterial pressure and coronary sinus oxygen saturation with the change in coronary arterial pressure always occurring significantly earlier than those for coronary sinus oxygen saturation. The half-time to greatest effect was not significantly different but the half-time for recovery was significantly longer for coronary arterial pressure because of the very marked vasodilator properties of this drug. The effect of ACh was blocked by atropine and was unaffected by propranolol.

Effect of vagal stimulation. In two experiments stimulation of the peripheral end of the cut left vagus caused a simultaneous fall in coronary

arterial pressure and bradycardia (Fig. 8*B*); this was followed by a decrease in aortic blood pressure which in turn led to an increase in coronary sinus oxygen saturation. This fall in coronary arterial pressure may have been due entirely to the bradycardia. A delayed rise in aortic pressure occurred which was probably reflex, secondary to the hypotension.

Effect of the intracoronary injection of hypertensin. The possibility existed that no response other than a fall in coronary arterial pressure could be elicited in the present experiments. This was ruled out because
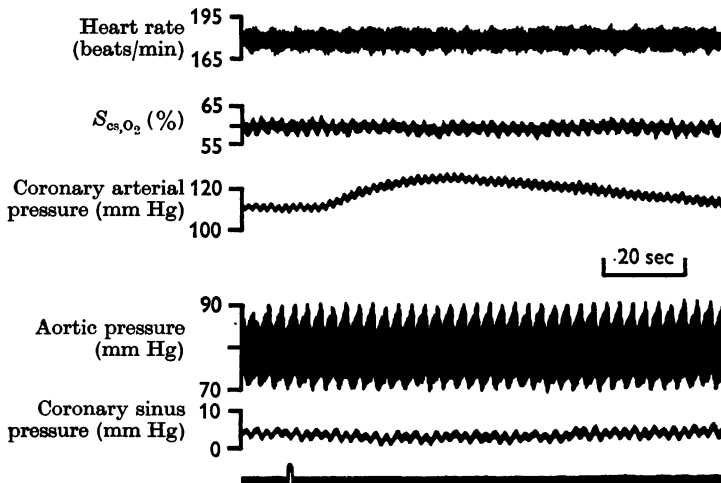

Fig. 9. Effect of the injection of hypertensin (0·01 μg) directly into the inflow at the signal. Notation as in Figs. 3, 5 and 8.

in ten experiments hypertensin injected into the coronary artery in doses of 0·01–0·1 μg always caused an increase in coronary arterial pressure (Fig. 9). Generally this was the only response evoked but in two experiments a small rise in aortic pressure occurred later. Bohr & Uchida (1967) have shown that hypertensin caused contraction of isolated strips of sheep coronary artery.

<div align="center">DISCUSSION</div>

Since stimulation of the peripheral ends of the cut Aδ fibres of cardiac sympathetic nerves had no effect on any of the parameters measured, the possibility of an axon reflex in the heart mediated by these particular nerve fibres could be ruled out. Stimulation of the preganglionic B fibres that run without synapse through the stellate ganglion was also ineffective, although occasionally a small rise of aortic pressure was elicited. This result differs from that of Juhász-Nagy & Szentiványi (1961) who showed that stimulation of certain post-ganglionic branches of the stellate ganglion

of dog and cat using stimulus parameters which were thought to excite only preganglionic fibres, evoked either coronary vasodilatation or vaso-constriction. The interpretation of their findings is made difficult because of the possibility that post-ganglionic sC fibres may have been stimulated as well. Moreover Gregg & Fisher (1963) were unable to repeat their results. If the preganglionic fibres that run through the stellate ganglion without relaying do synapse in or near the heart as has been suggested (Juhász-Nagy & Szentiványi, 1961; Napolitano, Willman, Hanlon & Cooper, 1967), they may be too few in number to have much effect. That their number is small appears likely from the small B elevation evoked in the post-ganglionic nerves by stimulation of the preganglionic trunk (Brown, 1967*b*).

The effects of stimulating the post-ganglionic sC fibres in the cardiac sympathetics and of intracoronary injections of noradrenaline were similar, as was to be expected (Euler, 1959). The fall in coronary arterial pressure which reflected coronary vascular resistance, was not due to a change in systolic ejection time. Nor was it due to a fall in intramyocardial tension because it occurred while the aortic pressure was rising, i.e. at a time when the intra-myocardial tension must have been increasing which in itself should have increased coronary resistance. In addition the fall in coronary resistance was even greater than was indicated by the changes of coronary arterial pressure alone for it occurred despite an increase of coronary sinus pressure.

There remains the question of the relation of the vasodilatation to the increased myocardial metabolism which resulted from the greater cardiac work evoked by noradrenaline. At constant inflow, the increased work caused a fall in coronary sinus oxygen saturation, which in turn reflected myocardial oxygen tension. The fall in coronary resistance began earlier than the fall in saturation and reached its nadir earlier. There was in fact no correlation between the half-times to maximum effect, that for coronary resistance being much faster. One possible explanation of these differences was an increase in coronary blood volume during sympathetic stimulation or noradrenaline injection which would have made the correction factor for the total lag time of coronary capillary oxygen saturation too small. Volume increases of four- to twenty-fold would have been required to account for the differences but in ten experiments there was no measurable change from control of either coronary blood volume or appearance time of the injected dye particles. Even though the indicator-dilution method is unreliable in the unsteady state, changes in volume of this order of magnitude should have been detected, (Zierler, 1958) and it is concluded that if changes in coronary blood volume did occur, they were much too small to account for the differences observed

The evidence, therefore, strongly suggests that the initial vasodilatation elicited by noradrenaline is independent of changes in myocardial metabolism. In the later part of the response, however, it is probable that coronary resistance was affected by decreased myocardial oxygen tension since a high degree of correlation between coronary sinus oxygen saturation and coronary resistance was present. It was also possible by using small doses of noradrenaline, to elicit substantial falls in coronary resistance with minimal changes in aortic blood pressure, cardiac output or coronary sinus oxygen saturation. Finally in the K^+-arrested heart, falls in coronary resistance were evoked without any change in saturation. These results strongly indicate that stimulation of the cardiac sympathetic nerves or noradrenaline act directly to cause vasodilatation of the coronary arteries of the cat. However, as pointed out above, noradrenaline can also produce vasodilatation indirectly through an increase in myocardial metabolism.

The effects of noradrenaline and of cardiac sympathetic stimulation were blocked by propranolol; vasodilatation evoked by ACh or stopping inflow was unaffected. On the other hand, atropine blocked the vasodilatation due to ACh without affecting the response to stimulation of the cardiac sympathetics, noradrenaline or greatly reduced inflow. This indicates that different receptor sites in the arteriolar smooth muscle were involved in the vasodilatation provoked by noradrenaline, acetylcholine or myocardial ischaemia. In the presence of propranolol, noradrenaline did not evoke vasoconstriction, indicating that α-receptors are not present in the coronary bed of the cat.

The interpretation of the present results differs from that given for dog. While the main effect of noradrenaline in that species is to cause coronary vasodilatation, this response has been attributed entirely to the concomitant increase of myocardial metabolism (Berne, 1964). It would seem, however, that it is important to establish the correlation in time between the changes of these two parameters before coming to this conclusion. This has not been done in the dog. However, there may be species differences from the cat as it is known that the coronary arteries of the dog appear to have some α-receptor sites (Berne, 1958; Gaal *et al.* 1966).

In two experiments, stimulation of the cardiac sympathetic nerves after both α- and β-adrenergic blockade had no effect on coronary resistance. Nor did atropine alter the vasodilatation evoked by such stimulation before blockade. Thus sympathetic cholinergic vasodilator fibres which innervate the arterioles of skeletal muscle (Eliasson, Folkow, Lindgren & Uvnäs, 1951) do not appear to supply the arterioles of cardiac muscle. Feigl (1967) was not able to demonstrate the presence of such nerve fibres in the dog.

Acetylcholine produced coronary vasodilatation which is in agreement

with the results of Berne (1958). The effects of vagal stimulation on coronary resistance were not definite because bradycardia occurred simultaneously with the fall in coronary resistance. Since the transmitter released during vagal stimulation is probably ACh, or less commonly, noradrenaline (Hoffmann, Hoffmann, Middleton & Talesnik, 1945), the results of the present study encompass any direct effects of vagal stimulation on the coronary vasculature.

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