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The pharmacological basis of coronary and systemic vasodilator actions of diazepam (Valium)

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Summary

1. The effects of α and β-adrenoceptor blockade, depletion of catecholamine stores, vagotomy, atropine, and ganglionic blockade on diazepam-induced vasodilatation were investigated in forty-six anaesthetized dogs.

2. Coronary blood flow was measured by timed collections of coronary venous efflux from fibrillating, decompressed ventricles; coronary and systemic vascular resistances were determined during total cardiopulmonary bypass under conditions of normothermia and constant aortic (coronary artery) pressure.

3. No significant alteration in the vasodilatation produced by diazepam was observed following either vagotomy or α-adrenoceptor blockade; partial inhibition of vasodilatation occurred after β-adrenoceptor blockade or catecholamine depletion, and nearly total inhibition was observed after small doses of atropine or ganglion-blocking agents.

4. The results suggest that diazepam may act as a specific ganglion-stimulant, causing active sympathetic and cholinergic vasodilatation.

Introduction

Previous experiments in this laboratory demonstrated that diazepam (Valium) decreases coronary vascular resistance (Abel, Reis & Staroscik, 1970). Although it was observed that these relationships are independent of extracardiac humoral mechanisms and appeared to require the delivery of diazepam to the coronary circulation, the mechanism by which diazepam produced coronary vasodilatation was not defined. A decrease in systemic vascular resistance also occurred consistently after diazepam administration, but the explanation for this phenomenon is likewise unclear. Chai & Wang (1966) studied the effects of diazepam on the nervous system of cats and demonstrated that diazepam decreased the medullary and hypothalamic pressor responses and decreased the cardiac accelerator response to stellate ganglion stimulation, in addition to manifesting some ganglion blocking effects. These investigators also noted decreased vascular resistance after injection of diazepam into the femoral artery of the sympathectomized hind limb of atropinized cats, suggesting a direct vasodilating effect of diazepam on blood vessels. A better understanding of the effects of diazepam on the autonomic nervous system may be helpful in defining the mechanism by which a psychotropic agent such as diazepam produces

the side effects of coronary and systemic vasodilatation, and it was for this reason that the experiments described in the following report were designed.

Methods

Forty-six unselected mongrel dogs of both sexes (weight 18–23 kg) were anaesthetized with intravenous sodium pentobarbital (35 mg/kg) and succinylcholine (1·0 mg/kg). An endotracheal tube was inserted, and respiration was controlled with a positive pressure ventilator supplying 100% oxygen. A bilateral trans-sternal thoracotomy was performed, and sodium heparin (3 mg/kg) was administered intravenously. The venae cavae and femoral artery were cannulated and cardiopulmonary bypass instituted with a Kay-Cross disc oxygenator and roller pump (Fig. 1). The extracorporeal circuits were primed with 3,500 ml of fresh, heparinized, homologous blood in normal saline (3:1 dilution). Blood temperature was monitored by a thermistor in the inferior vena cava and was maintained 37° C by a heat exchanger in the extracorporeal circuit. Blood gas tensions and the pH of arterial blood were determined periodically. Mean aortic and right atrial pressures were measured through indwelling catheters connected to Statham P23Db pressure transducers and continuously recorded at paper speeds of 0·25 mm/s with a direct-writing multi-channel oscillograph. All branches of the descending aorta were ligated to exclude extracoronary blood flow from entering the left atrium and to obviate extracoronary myocardial blood flow (Hudson, Moritz & Wearn, 1932). Coronary venous efflux was collected through large bore, multifenestrated catheters placed in both ventricles through the atria. Coronary blood flow was measured by timed volume collections of coronary venous efflux. The main pulmonary artery was ligated to ensure collection of all coronary blood returning to the right side of

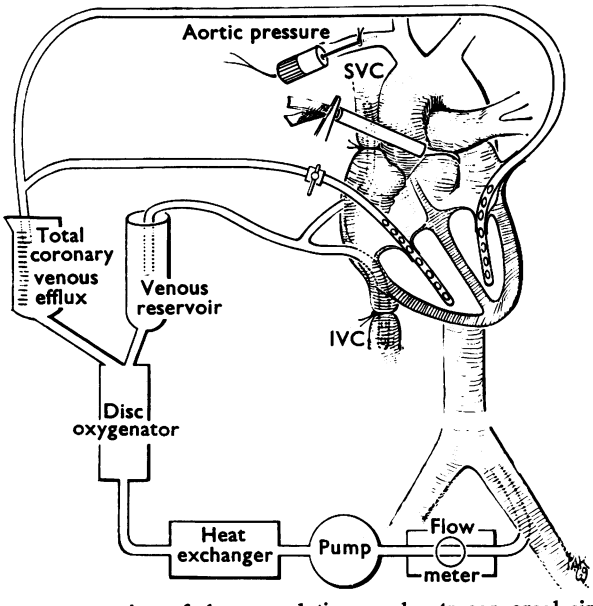

FIG. 1. Schematic representation of the cannulations and extracorporeal circuit used to assess coronary and systemic vascular resistances. Mean aortic pressure was maintained constant. Systemic blood flow (femoral artery inflow) was measured by an electromagnetic flow probe; coronary arterial flow was measured by timed volume collections of coronary venous efflux.

the heart. Ventricular fibrillation was induced by the brief application of an electrical stimulus to the right ventricle, and all subsequent determinations were performed during ventricular fibrillation. Systemic blood flow (femoral artery inflow) was measured by an electromagnetic flow probe in the line returning blood to the animal. A mean aortic pressure between 50 and 100 mmHg (1 mmHg≡1.333 mbar) was selected and maintained constant during each experiment by adjustment of the cardiopulmonary bypass flow rate as dictated by changes in systemic vascular resistance.

Systemic vascular resistance was calculated as

$$\frac{\text{Mean aortic pressure (mmHg)} - \text{mean right atrial pressure (mmHg)}}{\text{Systemic blood flow (litre/min)} - \text{coronary venous efflux (litre/min)}}$$

and expressed in resistance units (R.U.).

Coronary vascular resistance was calculated as

$$\frac{\text{Mean aortic (coronary artery) pressure (mmHg)} - \text{mean right atrial pressure (mmHg)}}{\text{Coronary venous efflux (ml/min)}} \times 100$$

and expressed in coronary resistance units (C.R.U.).

A stable control state was established before each experiment, and during this period variation in coronary blood flow did not exceed ±5%. Diazepam (0·2 mg/kg) was then injected into the arterial side of the oxygenator as mean aortic and right atrial pressures, and coronary and systemic blood flows were measured. A second injection of diazepam (1·0 mg/kg) was given 10 min after the initial dose, and pressures and flows were measured for an additional 10 min period. During the entire experiment, mean aortic pressure was maintained by controlling pump flows. Systemic and coronary vascular resistances were calculated before, and 1, 2, 5, and 10 min after each injection of diazepam. The animals were divided into seven groups by the interventions which preceded diazepam administration.

The significance of changes in resistance after diazepam in each group of animals, compared to control values, was determined by a one-tailed Student's *t* test for unpaired groups of data using an equal variance model. Following each experiment both ventricles were weighed and coronary blood flow expressed as ml/100 g ventricle per min.

Group I: normal dogs

In nine animals systemic and coronary vascular resistances were calculated before and after diazepam.

Group II: β-adrenoceptor blockade

In seven dogs propranolol (1 mg/kg) was administered intravenously 10 min before cardiopulmonary bypass, and an additional 1 mg/kg was given 10 min after instituting bypass. All observations were completed within 1 h after the initial propranolol dose. After each diazepam experiment, a dose-response curve to isoprenaline was inscribed to determine the efficacy of β-adrenoceptor blockade. Sequential injections of 0·1, 1, 5, 10, 50, 100 and 200 μg isoprenaline were made into the arterial end of the oxygenator as peak changes in systemic and coronary vascular resistance were determined.

Group III : α-adrenoceptor blockade

Phenoxybenzamine HCl (2·5–7·5 mg/kg) was injected into the oxygenator immediately after the institution of cardiopulmonary bypass in five dogs. After each experiment the effects of phenylephrine (1, 5, 10, and 50 mg) on systemic and coronary vascular resistances were determined.

Group IV : reserpine pretreatment

Ten animals received intramuscular reserpine (2 mg/kg) 24 h before each experiment and 500 ml lactated Ringers solution intravenously approximately 12 h later. Four of these dogs died before studies were carried out, but the effects of diazepam on systemic and coronary vascular resistances were determined in the remaining six. In each animal, the changes in coronary and systemic vascular resistances produced by tyramine hydrochloride (500, 1,000, and 2,000 μg) were determined at the conclusion of each experiment.

Group V : vagotomy

In seven dogs, bilateral cervical vagotomy was performed just before cardiopulmonary bypass. The effects of diazepam on systemic and coronary vascular resistances were then determined.

Group VI : cholinoceptor (muscarinic) blockade

In six dogs atropine (2·0 mg/kg) was injected into the oxygenator just after institution of extracorporeal circulation. The effects of diazepam were then determined in these animals.

Group VII : ganglion blockade

Continuous intravenous infusion of trimethaphan camphorsulphonate (Arfonad) (10–20 mg/min) was initiated just after institution of cardiopulmonary bypass in seven dogs. The effects of diazepam on systemic and coronary vascular resistances were then determined. At the conclusion of each experiment, and while trimethaphan infusion was continued, 100 μg isoprenaline was injected into the oxygenator, or profound systemic hypoxaemia was produced, and the effects on systemic and coronary vascular resistances determined.

Results

The mean coronary and systemic vascular resistances determined during the control period in each of the seven groups are listed in Table 1. The % changes, compared with control values of coronary and systemic vascular resistances in each group after diazepam, are summarized in Tables 2 and 3.

Group I : normal dogs

Significant decreases in coronary vascular resistance were evident 1, 2, and 5 min after 0·2 mg/kg diazepam. Although decreases persisted 10 min after diazepam, they were not statistically significant at that time. Similar changes of greater magnitude occurred after 1·0 mg/kg diazepam. Significant decreases in systemic vascular resistance were evident after diazepam at each evaluation period, and were more marked after the larger dose (Fig. 2).

Group II : β-adrenoceptor blockade

Average coronary vascular resistance in the control state was slightly less than the average control vascular resistance in the group of normal dogs. There was no change in systemic or coronary vascular resistance after 0·2 mg/kg diazepam. Decreases in coronary and systemic vascular resistances occurred after 1·0 mg/kg diazepam, but these changes were not as marked as those observed in normal dogs (Group I) (Fig. 3A and B).

No changes in systemic or coronary vascular resistance resulted from the injection of isoprenaline in doses of 50 μg or less. One hundred and 400 μg isoprenaline produced very small changes in coronary and systemic vascular resistances compared with the changes produced by the same dose in the group of normal dogs. Representative isoprenaline and diazepam dose-response curves from a normal dog (Group I) and from a dog (Group II) (4) with β-adrenoceptor blockade are reproduced in Fig. 4.

Group III : α-adrenoceptor blockade

Mean coronary and systemic vascular resistances determined during the control period were significantly less than the respective average control resistances in the group of normal dogs ($P<0·05$). Significant additional decreases in coronary vascular resistance occurred after 0·2 mg/kg or 1·0 ml/kg diazepam was administered ; 0·2 mg/kg diazepam produced no change in systemic vascular resistance, but significant decreases did occur after 1·0 mg/kg diazepam (Fig. 3C and D).

A comparison of the changes occurring after diazepam in this group of dogs and in the control group of animals revealed no significant differences except for the changes in systemic vascular resistance which occurred 1 and 5 min after 0·2 mg/kg diazepam, when significantly less decreases in systemic resistance were evident in the dogs with α-adrenoceptor blockade.

TABLE 1. *Coronary and systemic vascular resistance during the control period in seven groups of animals*

Group	No. of animals	Coronary vascular resistance (C.R.U. ± S.E.M.)	Systemic vascular resistance (R.U. ± S.E.M.)
I Normal	9	741·8 ± 99·0	57·2 ± 4·7
II β-adrenoceptor blockade	7	661·5 ± 48·6 (NS)*	57·1 ± 7·3 (NS)
III α-adrenoceptor blockade	5	519 ± 60·4 ($P<0·05$)	27·6 ± 4·9 ($P<0·0025$)
IV Catecholamine depletion	6	639 ± 73·7 (NS)	55·8 ± 5·3 (NS)
V Vagotomy	7	741 ± 98·3 (NS)	60·0 ± 8·1 (NS)
VI Cholinoceptor blockade (Atropine)	6	566·5 ± 86·5 (NS)	41·1 ± 6·0 ($P<0·05$)
VII Ganglion blockade (trimethaphan)	7	379·8 ± 46·8 ($P<0·005$)	24·7 ± 2·0 ($P<0·005$)

* Statistical comparisons between the mean resistance in each group compared with the mean resistance in normal dogs (group 1).
R.U., Resistance units. NS, not significant ($P>0·1$).

TABLE 2. *Coronary vascular resistance changes after diazepam*

Group	No. of animals	Mean control coronary blood flow (ml/100 g)/min	Change in coronary vascular resistance (% of control ± S.D.)							
			Time (min) after diazepam 0·2 mg/kg				Time (min) after diazepam 0·1 mg/kg			
			1	2	5	10	1	2	5	10
I Normal	9	88	−13·9 ±12·4	−12·4 ±13·1	− 5·8 ±7·6	−4·0 ±8·0	−31·0 ±17·9	−37·5 ±19·4	−23·4 ±14·0	−21·5 ±16·4
II β-adrenoceptor blockade	7	72	−1·3 ±1·9 (P^*<0·025)	−0·9 ±2·0 P<0·01	8·3 ±7·7 P<0·005	11·5 ±11·9 P<0·01	−20·1 ±14·5 NS	−14·8 ±9·0 P<0·0125	−8·2 ±9·7 P<0·05	−8·2 ±9·7 NS
III α-adrenoceptor blockade	5	83	−6·3 ±4·1 NS	−13·6 ±18·3 NS	−8·0 ±6·8 NS	−11·5 ±8·1 NS	−17·3 ±12·6 NS	−22·6 ±17·5 NS	−12·2 ±6·0 NS	−10·0 ±4·9 NS
IV Catecholamine depletion	6	97	−4·5 ±5·4 P<0·05	0·9 ±4·6 P<0·01	4·3 ±4·1 P<0·005	4·8 ±6·2 P<0·025	−22·1 ±10·3 NS	−18·3 ±8·0 P<0·025	−13·3 ±24·2 NS	−13·7 ±8·4 NS
V Vagotomy	7	87	−9·2 ±5·8 NS	−6·0 ±4·2 NS	−0·9 ±3·3 NS	−1·6 ±6·6 NS	−26·2 ±15·3 NS	−28·2 ±18·1 NS	−17·8 ±5·4 NS	−17·8 ±16·3 NS
VI Cholinoceptor blockade	6	89	−3·4 ±2·9 P<0·025	−1·6 ±1·0 P<0·025	−1·7 ±4·3 P<0·025	5·6 ±7·9 P<0·05	−9·7 ±8·4 P<0·0125	−10·8 ±6·3 P<0·005	−7·9 ±8·0 P<0·05	−1·2 ±10·7 P<0·025
VII Ganglion blockade	7	90	−2·7 ±3·0 P<0·025	−1·0 ±4·1 P<0·01	−2·7 ±5·9 NS	−0·4 ±8·1 NS	−4·3 ±4·7 P<0·005	−4·8 ±4·0 P<0·0025	−3·0 ±2·8 P<0·0125	1·8 ±6·7 P<0·0125

* Significance levels determined by comparing the % change from control resistance at 1, 2, 5, and 10 min after diazepam and changes at similar intervals in normal animals (group 1).
NS, Not significant (P>0·1).

TABLE 3. *Changes in systemic vascular resistance following intravenous diazepam*

Group	No. of animals	Mean control systemic blood flow ((ml/kg)/min)	Change in systemic vascular resistance (% of control ±S.D.)							
			Time (min) after diazepam 0·2 mg/kg				Time (min) after diazepam 0·1 mg/kg			
			1	2	5	10	1	2	5	10
I Normal	9	74	−30·0 ±8·4	−3·8 ±4·3	−9·4 ±5·4	−12·3 ±10·4	−51·1 ±11·5	−39·5 ±23·7	−26·8 ±18·5	−24·2 ±19·0
II β-adrenoceptor blockade	7	68	−13·3 ±20·1 NS	0·9 ±3·1 P*<0·0125	2·0 ±6·7 P<0·0025	5·0 ±8·6 P<0·0025	−39·5 ±22·2 NS	−4·8 ±3·4 P<0·05	−8·7 ±4·9 P<0·05	−13·0 ±3·0 NS
III α-adrenoceptor blockade	5	88	−12·9 ±14·1 P<0·05	−10·3 ±27·8 NS	1·8 ±5·2 P<0·005	2·1 ±15·3 NS	−41·0 ±21·3 NS	−29·3 ±26·8 NS	−12·0 ±3·5 NS	−8·0 ±4·4 NS
IV Catecholamine depletion	6	79	−12·1 ±12·6 P<0·025	1·5 ±12·0 NS	−6·4 ±6·6 NS	−11·1 ±6·4 NS	−25·1 ±17·2 P<0·025	−14·0 ±7·8 P<0·025	−11·0 ±6·3 P<0·05	−12·7 ±8·5 NS
V Vagotomy	7	70	−34·3 ±15·5 NS	−1·8 ±5·6 NS	−6·2 ±6·5 NS	−7·1 ±13·5 NS	−56·8 ±13·4 NS	−23·7 ±27·8 NS	−22·6 ±24·3 NS	−26·1 ±22·2 NS
VI Cholinoceptor blockade	6	84	−6·3 ±9·7 P<0·005	5·8 ±7·9 P<0·025	4·0 ±3·5 P<0·0005	9·3 ±10·1 P<0·0025	−25·1 ±16·0 P<0·005	−4·7 ±7·8 P<0·005	−9·2 ±6·5 P<0·05	−3·2 ±9·3 P<0·025
VII Ganglion blockade	7	100	−0·8 ±2·3 P<0·0025	−0·1 ±2·6 P<0·05	1·5 ±3·6 P<0·0005	3·8 ±6·3 P<0·0025	−8·2 ±5·3 P<0·0005	−6·0 ±4·9 P<0·01	−5·3 ±5·9 P<0·025	0·5 ±12·6 P<0·025

* Significance levels determined by comparing the % change from control resistance at 1, 2, 5, and 10 min after diazepam with changes at similar intervals in normal animals (group 1).
NS, Not significant ($P>0·01$).

No changes in systemic or coronary vascular resistances occurred following the injection of phenylephrine in these dogs, except in two instances when slight decreases in coronary vascular resistance were observed after 10 and 50 mg doses of phenylephrine.

Group IV : reserpine pretreatment

All animals had resting heart rates less than 60 beats/min and average control coronary and systemic vascular resistances, although somewhat less, were not significantly different from the resistances observed in normal dogs. The responses following diazepam were similar to those in dogs with β-adrenoceptor blockade. No significant changes in systemic or coronary vascular resistance were evident after 0·2 mg/kg diazepam. Decreases in coronary and systemic vascular resistances occurred after 1·0 mg/kg diazepam, but these changes were not as great as those observed in normal dogs (Fig. 3E and F).

The injection of 500 μg tyramine hydrochloride produced no change in systemic or coronary vascular resistances ; small decreases in resistance (5–15%) were evident after 1,000 and 2,000 μg doses.

Group V : vagotomy

There were no significant differences in average systemic or coronary vascular resistances determined in the control state or after diazepam in these dogs compared to the average resistances observed in the group of normal dogs (Fig. 5A and B).

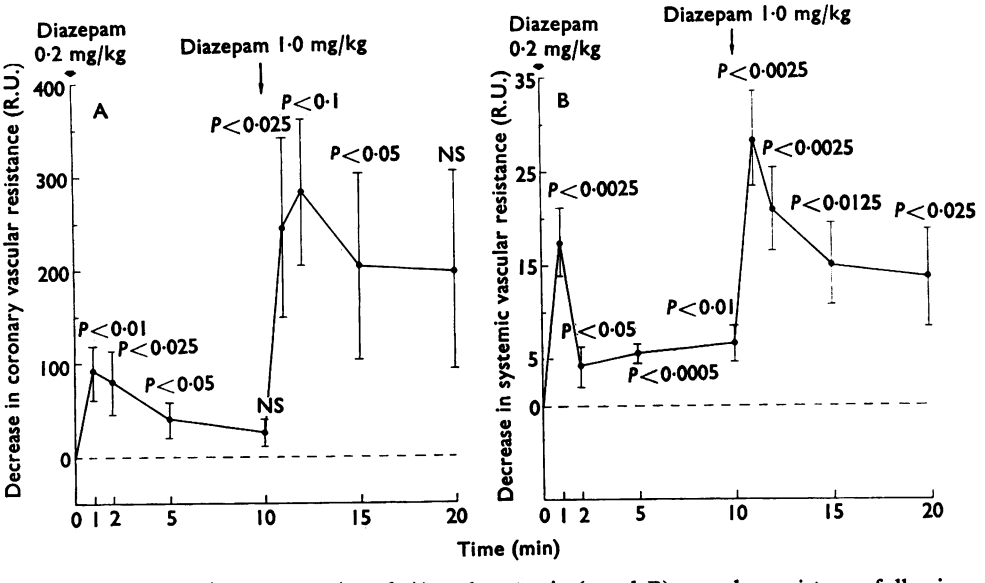

FIG. 2. Decreases in coronary (panel A) and systemic (panel B) vascular resistance following diazepam 0·2 mg and 1·0 mg/kg in nine normal dogs. The values shown represent the mean change ; the vertical brackets indicate the standard error of the mean change. The statistical significance of each value is indicated. Statistical analyses were performed comparing the values at 1, 2, 5, and 10 min following diazepam and values determined during the control period.

Group VI: cholinoceptor (muscarinic) blockade

Average control coronary and systemic vascular resistances were somewhat less in animals with cholinoceptor (muscarinic) blockade than the average control vascular resistances in normal dogs, but these differences were not statistically significant. No changes occurred in coronary or systemic vascular resistance after 0·2 mg/kg diazepam, except for a small decrease in coronary vascular resistance

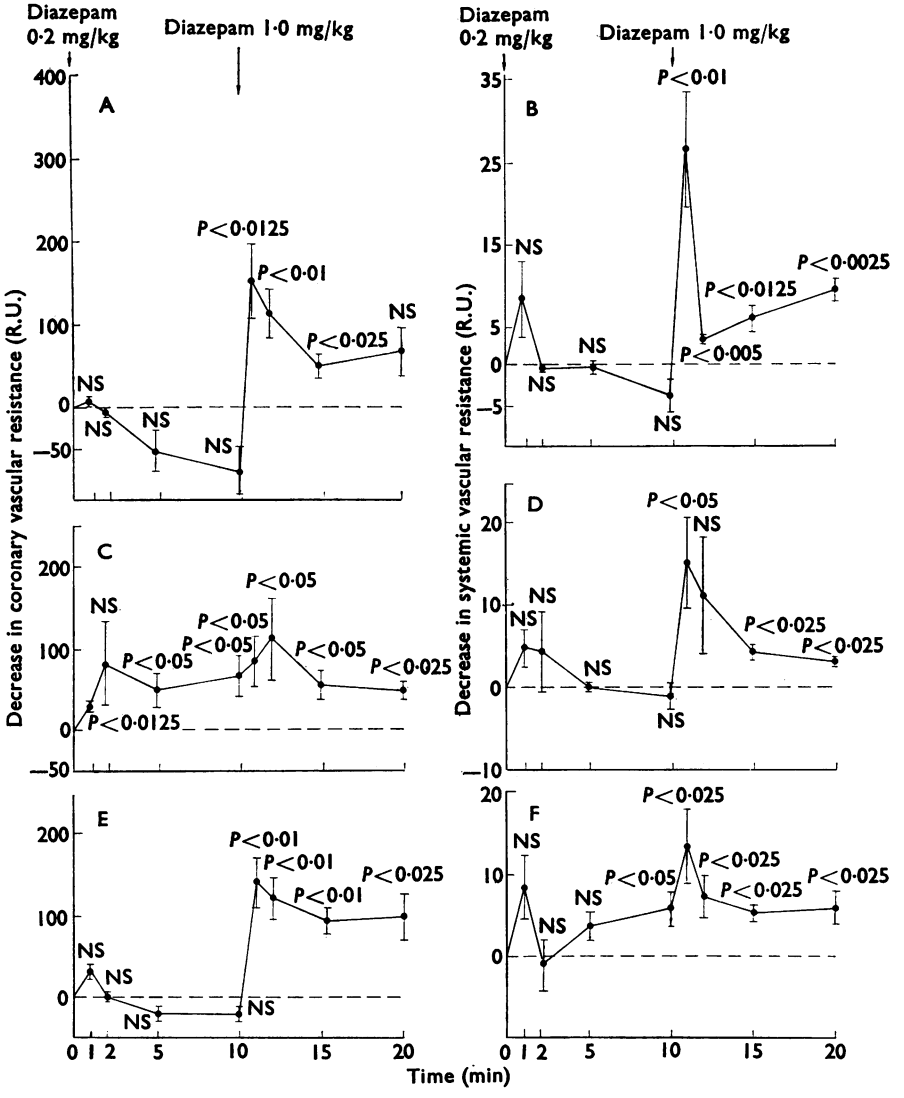

FIG. 3. Decreases in coronary and systemic vascular resistance following diazepam 0·2 mg/kg and 1·0 mg/kg. Panels A and B, animals with β-adrenoceptor blockade; panels C and D, animals with α-adrenoceptor blockade; panels E and F, animals with reserpine pretreatment. *P* values for statistical evaluation were obtained from the mean of individual decreases in coronary and systemic vascular resistance compared to control values at each point in time. NS, Not significantly different from control. Vertical brackets indicate the standard error of the mean decreases.

evident 1 and 2 following the drug. Decreases in coronary and systemic vascular resistances occurred after 1·0 mg/kg diazepam, but these changes were not as marked as those observed in normal dogs (Fig. 5C and D).

Group VII : ganglion blockade

Trimethaphan infusion produced marked decreases in systemic and coronary vascular resistances, and resistances during the control period were significantly less than in normal dogs ($P<0·005$). There were no changes in coronary or systemic vascular resistances in these animals after 0·2 mg/kg diazepam.

Small decreases in systemic and coronary vascular resistance occurred after 1·0 mg/kg diazepam, but the magnitude of these changes was significantly less than that observed after the administration of diazepam to normal dogs (Fig. 5E and F).

At the conclusion of each experiment and while trimethaphan administration was continued, isoprenaline or systemic hypoxaemia produced significant further decreases in coronary and systemic vascular resistance.

Discussion

It has previously been demonstrated that diazepam produces coronary vasodilatation following administration to the neurally intact heart isolated from the systemic circulation, but that systemic administration in this preparation does not produce coronary vasodilatation (Abel *et al.*, 1970). These findings suggested that the decrease in coronary vascular resistance might be due to direct effects of the drug on the coronary arteries, but the mechanisms by which this occurred were not elucidated. In the present study, interactions of diazepam with autonomic control of vascular tone in the systemic circulation and in one specific vascular bed, the myocardial circulation, were investigated in an attempt to define the mechanisms by which diazepam causes vasodilatation.

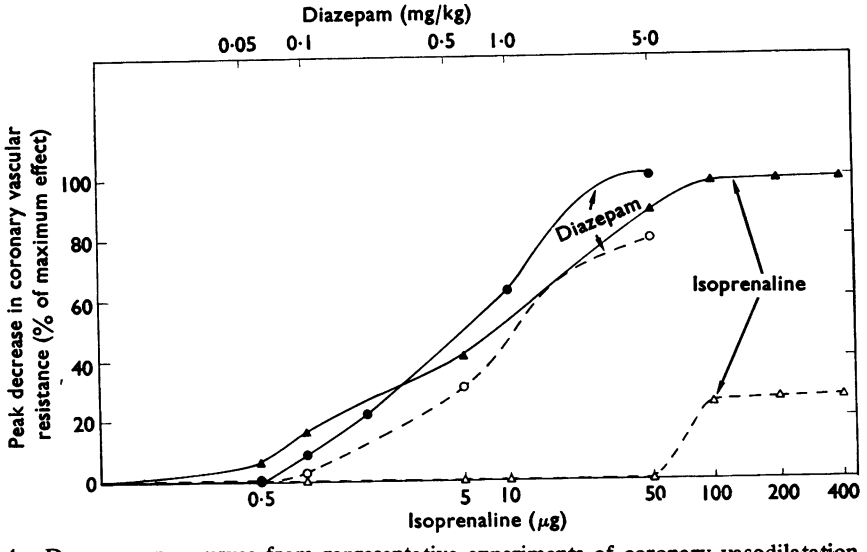

FIG. 4. Dose-response curves from representative experiments of coronary vasodilatation produced by diazepam (upper abscissa) 0·01 mg/kg to 5·0 mg/kg and isoprenaline (lower abscissa) 0·5 μg to 400 μg given as bolus injections. 100% vasodilatation was that achieved with the highest dose of each agent. – – –, β-adrenoceptor blockade ; ——, normal.

Groups II, III, and IV (β-adrenoceptor blockade, α-adrenoceptor blockade, and catecholamine depletion) examined possible adrenergic vasodilating mechanisms. Partial inhibition of diazepam-induced coronary and systemic vasodilatation by β-adrenoceptor blockade with propranolol suggested that diazepam acts either directly or indirectly at the β-adrenoceptor sites. Larger doses of diazepam still produced significant decreases in vascular resistance, but to a lesser degree than in normal animals. The doses at which diazepam elicited vascular responses and the peak diazepam-induced vasodilatation achieved were less than in normal dogs and paralleled depression of isoprenaline-induced coronary vasodilatation during β-adrenoceptor blockade. Competitive inhibition by propranolol may explain this dose-response relationship.

Since diazepam may have acted by releasing catecholamines at post-ganglionic adrenergic nerve fibres, rather than having a direct stimulant action on β-adrenoceptor sites, the group of animals pretreated with reserpine was investigated to differentiate between these two possible mechanisms. The partial inhibition of vasodilatation observed following diazepam in these animals was of the same mag-

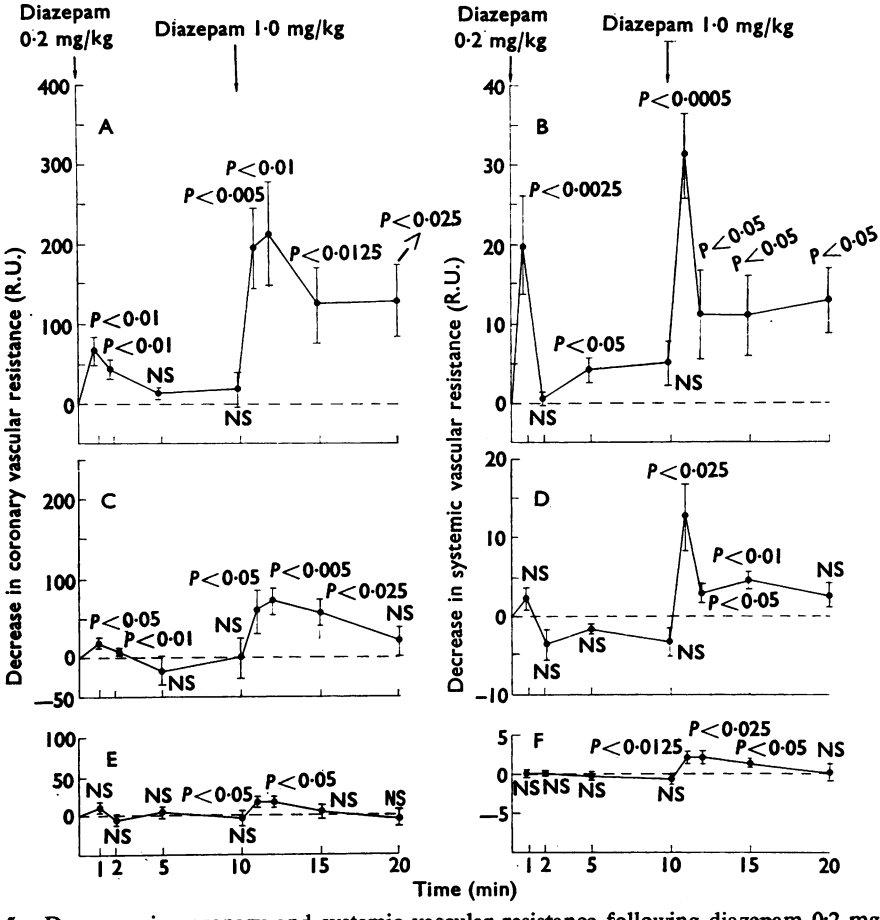

FIG. 5. Decreases in coronary and systemic vascular resistance following diazepam 0·2 mg/kg and 1·0 mg/kg. Panels A and B, animals with cervical vagotomy; panels C and D, animals with muscarinic blockade (atropine); panels E and F, animals with ganglion blockade trimethaphan.

nitude as that observed in the group with β-adrenoceptor blockade. The minimal responses in these animals to large doses of tyramine, compared with normals, however, suggests that total depletion of catecholamines was not achieved and, in addition, adrenalectomy was not performed. The vasodilatation produced by large doses of diazepam in this group of dogs was less than in normal dogs and could have been due to the release of noradrenaline at adrenergic nerve endings.

The possibility that an alternative adrenergic mechanism was responsible for the vasoactive responses following diazepam was investigated by producing α-adrenoceptor blockade. Ablation of the predominant alpha-constrictor tone in the coronary arteries and hence " unmasking " β-adrenoceptor vasodilatation influences is a possible mechanism for coronary vasodilatation, but such a mechanism would not necessarily explain systemic vasodilatation, as the response of the systemic vascular bed to α-adrenoceptor blockade is dependent on the resting " tone " of the sympathetic nervous system. The coronary and systemic vasodilatation produced by diazepam was not significantly altered by α-adrenoceptor blockade, however, suggesting that this mechanism was not responsible for the vasoactive effects of diazepam.

Groups V and VI (vagotomy and atropine) attempted to define whether diazepam acted through parasympathetic vasodilating pathways. The demonstration that bilateral cervical vagotomy does not influence the coronary vasodilatation produced by diazepam places the parasympathetic site of action, if any, distal to efferent preganglionic vagal fibres to the myocardium.

The dose of atropine given to the dogs in group VI would be expected to provide blockade of the muscarinic, but not nicotinic (ganglion-blocking), effects of acetylcholine. Atropine significantly, but not completely, blocked both the coronary and

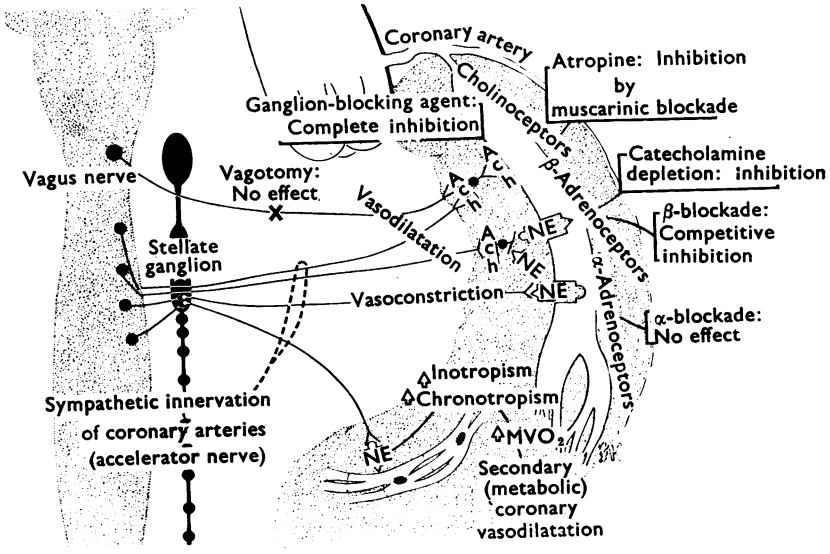

FIG. 6. Proposed mechanisms of vasomotor actions of diazepam. The shaded area to the right represents the myocardium, the clear area in the centre of which designates a coronary artery. The shaded area to the left represents the central nervous system. Stimulation of postganglionic fibres within the ventricular myocardium or in the coronary arteries themselves is the presumed site of action of diazepam.

peripheral vasodilating actions of diazepam, suggesting that a cholinergic vasodilating mechanism may also be involved. Since the vagotomy experiments established that the vagus-blocking effects of atropine were irrelevant, and since the dose of atropine used should not have produced significant ganglion-blockade, stimulation of postganglionic parasympathetics by diazepam remains a strong possibility for an explanation of the atropine-induced inhibition.

The most potent inhibitor of both the coronary and systemic vasodilatation produced by diazepam in the current groups of experiments was the ganglion-blocking agent, trimethaphan. The failure of diazepam to produce additional vasodilatation during trimethaphan administration could be explicable if maximal vasodilatation was elicited by trimethaphan, such that another stimulus could not produce further vascular relaxation. This was not the case, however, as severe hypoxaemia or catecholamine administration produced additional coronary vasodilation in these animals.

Although the autonomic supply to many peripheral vascular beds has been well defined, the neurophysiology of the coronary vascular bed remains a subject for continuing investigation. Although Klocke, Kaiser, Ross & Braunwald (1965) demonstrated an intrinsic adrenergic vasodilator mechanism in canine coronary arteries, there have been few specific demonstrations of neural control of dilator β-adrenoceptors in the coronary circulation. Szentivanyi & Juhasz-Nagy (1959) separated the "accelerator nerves" to the heart into at least three distinct fibres, stimulation of each producing either vasoconstriction, vasodilatation without changes in myocardial oxygen consumption or contractility, and vasodilatation secondary to augmentation of heart rate and contractility. Those fibres which produced only coronary vasodilatation were identified as cholinergic (Juhasz-Nagy & Szentivanyi, 1961) and were observed to "pass through" the stellate ganglion without synapsing, presumably connecting locally within the wall of the heart. Additional evidence of postganglionic sympathetic connections of canine cardiac nerves residing within or near the heart was recently provided by Wechsler, Pace, Goldberg & Randall (1969). Folkow, Frost, Haeger & Uvnas (1948) recovered an acetylcholine-like substance from coronary sinus blood following stellate ganglion stimulation in both dogs and cats, which also suggested that cholinergic fibres are carried to the myocardial circulation by the sympathetics. Although Schreiner, Berglund, Borst & Monroe (1957) demonstrated that vagus stimulation did not affect coronary blood flow in the paced canine heart, Daggett, Nugent, Carr, Powers & Harada (1967) observed significant increases in coronary blood flow following vagal stimulation in dogs in which ventricular work and aortic pressure were maintained constant.

Using anaesthetized dogs with blocked β-adrenoceptors, Feigl (1967) was unable to demonstrate coronary sympathetic cholinergic vasodilatation similar to that found in skeletal muscle. The existence of any significant tonic control of coronary blood flow, attributable either to sympathetic or parasympathetic neural pathways, is also doubted by other workers (Gregg & Fisher, 1963).

A hypothesis for the actions of diazepam on the coronary circulation based on the results of the present experiments is diagrammatically illustrated in Fig. 6. These actions are based on the neurophysiological understanding that the pathways demonstrated by Juhasz-Nagy & Sventivanyi (1961) of preganglionic sympathetics to the heart synapsing in ganglia within the myocardium or coronary arteries provide a significant basis for the control of coronary blood flow. Diazepam seems to

stimulate specifically vasodilating mechanisms (both adrenergic and cholinergic) at the post-ganglionic neurone and thereby affects coronary vascular resistance. The observations regarding changes in systemic vascular resistance suggest the possibility that similar mechanisms may also be responsible for the peripheral actions of diazepam.

REFERENCES

ABEL, R. M., REIS, R. L. & STAROSCIK, R. N. (1970). Coronary vasodilatation following diazepam (Valium). *Br. J. Pharmac.*, **38**, 620–631.

CHAI, C. Y. & WANG, S. C. (1966). Cardiovascular actions of diazepam in the cat. *J. Pharmac. exp. Ther.*, **154**, 271–280.

DAGGETT, W. M., NUGENT, G. C., CARR, P. W., POWERS, P. C. & HARADA, Y. (1967). Influence of vagal stimulation on ventricular contractility, 02 consumption, and coronary flow. *Am. J. Physiol.*, **212**, 8–18.

FEIGL, E. O. (1967). Sympathetic control of coronary circulation. *Circulation Res.*, **20**, 262–271.

FOLKOW, B., FROST, J., HAEGER, K. & UVNAS, B. (1948). Cholinergic fibres in the sympathetic outflow to the heart in the dog and cat. *Acta physiol. scand.*, **15**, 421–426.

GREGG, D. E. & FISHER, L. C. (1963). Blood supply to the heart. In *The Handbook of Physiology*, Section 2, Circulation, vol. 2, pp. 1517, 1584. Baltimore: The Williams and Wilkins Company.

HUDSON, C. L., MORITZ, A. R. & WEARN, J. T. (1932). The extracardiac anastomoses of the coronary arteries. *J. exp. Med.*, **56**, 919–931.

JUHASZ-NAGY, A. & SZENTIVANYI, M. (1961). Separation of cardiaccelerator and coronary vasomotor fibres in the dog. *Am. J. Physiol.*, **200**, 125–129.

KLOCKE, F. J., KAISER, G. A., ROSS, J., JR. & BRAUNWALD, E. (1965). An intrinsic adrenergic vasodilator mechanism in the coronary vascular bed of the dog. *Circulation Res.*, **16**, 376–382.

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

SZENTIVANYI, M. & JUHASZ-NAGY, A. (1959). A new aspect of the nervous control of the coronary blood vessels. *Q. J. exp. Physiol.*, **44**, 67–79.

WECHSLER, J. S., PACE, J. B., GOLDBERG, J. M. & RANDALL, W. C. (1969). Location of synaptic connections in canine sympathetic cardiac innervation. *Am. J. Physiol.*, **217**, 1789–1794.

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