Br. J. Pharmac. (1986), **87**, 265–277

The role of catecholamines in the production of ischaemia-induced ventricular arrhythmias in the rat *in vivo* and *in vitro*

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1 The role of catecholamines in the production of ischaemia-induced ventricular arrhythmias *in vivo* and *in vitro* was studied using coronary artery ligation in the rat.

2 Increases in plasma catecholamine concentrations during coronary artery ligation in pentobarbitone-anaesthetized animals were prevented by either acute adrenalectomy or chronic adrenal demedullation, but these procedures did not protect against the occurrence of ventricular arrhythmias. Thus plasma catecholamines were not obligatory mediators of arrhythmogenesis.

3 Three protocols were used *in vitro* to evaluate the possible influence of intramyocardial release of noradrenaline, produced by the local conditions of ischaemia, on the production of ventricular arrhythmias. During coronary artery ligation in isolated perfused hearts, no enhanced output of ^3H could be detected from [^3H]-noradrenaline loaded hearts, even in the presence of inhibitors of catecholamine uptake processes, although washout of lactate from ischaemic regions was readily demonstrable.

4 Both optical isomers of propranolol were equally effective in reducing the incidence of arrhythmias, implying a non-specific effect, since the (+)-isomer possesses considerably less β-adrenoceptor blocking activity. The equipotency of optical isomers of propranolol combined with a lack of effect of atenolol suggested that arrhythmia production was not a consequence of β-adrenoceptor stimulation.

5 The α-adrenoceptor blockers phentolamine and prazosin, both exerted antiarrhythmic actions of similar potency, but phenoxybenzamine and trimazosin had no significant effects. An evaluation of the pharmacological properties of the α-adrenoceptor blockers showed that those drugs which had demonstrable local anaesthetic properties also exerted significant antiarrhythmic effects. No relationship was found between potency of α-adrenoceptor blockade and antiarrhythmic efficacy.

6 The overall conclusion from these multifaceted approaches was that catecholamines were not necessary mediators of the early phase of ventricular arrhythmias in the rat.

Introduction

Disturbances of the hormonal and autonomic nervous systems have been observed during the early phase of acute myocardial ischaemia produced by coronary artery ligation (Gillis, 1971; Ceremuzynski, 1981). Attention has been drawn to the possible role of plasma catecholamine concentrations in the production of ventricular arrhythmias in addition to release of noradrenaline within the ischaemic myocardium.

The severity of ventricular arrhythmias produced in the early phase of acute myocardial ischaemia in the dog has been related to increased plasma concentrations of catecholamines (Ceremuzynski *et al.*, 1969) released from adrenal medullae (Kelliher *et al.*, 1975). Despite reports on detrimental effects of elevated plasma catecholamine concentrations, other studies have demonstrated a reduction of ventricular arrhythmias by increasing plasma concentrations of adrenaline (Marshall *et al.*, 1981) and noradrenaline (Regan *et al.*,1970; Parratt *et al.*, 1981). The present study has attempted to clarify the role of plasma catecholamines on the early phase of ischaemically-induced ventricular arrhythmias, using coronary artery ligation in

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rats in which plasma concentrations of catecholamines were greatly reduced by either chronic adrenal demedullation or acute adrenalectomy.

The present study also investigated the possible influence of intramyocardial noradrenaline release, caused by conditions within ischaemic cardiac tissue, on the production of ventricular arrhythmias. Release of noradrenaline within ischaemic myocardium has been found previously (Shahab *et al.*, 1972), although more recent studies have failed to demonstrate this phenomenon (McGrath *et al.*, 1981; Riemersma & Forfar, 1981). Many investigations have used pharmacological means to define whether the intramyocardial-release of catecholamines has an arrhythmogenic effect. However, it should be emphasized that the protective effect noted in some of these studies requires careful interpretation, since conditions prevailing within ischaemic myocardium alter pharmacological profiles of drugs, particularly with respect to enhancement of local anaesthetic properties (Vaughan Williams, 1978). To investigate whether noradrenaline is released directly by ischaemia and contributes to the production of ventricular arrhythmias, we have used a paradigm involving coronary artery ligation in the rat isolated heart. A rat isolated heart preparation has the advantage of being devoid of haemodynamic, hormonal and extrinsic neural influences with could obscure the interpretation of results. Preliminary accounts of this work have been presented previously (Daugherty & Woodward, 1982; Daugherty *et al.*, 1983).

Methods

In vivo *studies*

Coronary artery ligation in anaesthetized rats Coronary artery ligation was performed in anaesthetized rats by a method similar to that described by Clark *et al.* (1980), except that the heart was not exteriorized for the placement of suture. Arrhythmic activity was recorded for 30 min post-ligation. Parameters recorded were: premature ventricular contractions (PVCs), percentage incidence and duration of ventricular tachycardia (VT), percentage incidence and duration of venricular fibrillation (VF) and percentage mortality attributable to ventricular fibrillation.

Approximately 3 ml of arterial blood was removed by cardiac puncture after 30 min of coronary artery ligation. All blood samples were heparinized, centrifuged, and the plasma stored at $-20°C$ until catecholamine determinations were performed.

Determination of plasma catecholamines Plasma catecholamine concentrations were determined using the method of Frayn & Maycock (1983). Briefly, plasma was extracted with an ion-exchange column and alumina and the eluate was used for analysis. Adrenaline, noradrenaline and dopamine were resolved by reverse phase ion-pair high pressure liquid chromatography and the catecholamine concentrations were determined by electrochemical detection.

Adrenal demedullation and adrenalectomy procedures Adrenal demedullation was performed on male Wistar rats (60–90 g) under ether anaesthesia. Rats were given physiological saline to drink during the first post-operative week to compensate for possible temporary impairment of mineralocorticoid synthesis. Thereafter, they were given normal drinking water. Rats were subjected to coronary artery ligation when greater than 250 g (5 to 6 weeks after demedullation).

Acute adrenalectomy was performed on pentobarbitone-anaesthetized rats before cannulation and tracheotomy. Adrenal glands were exposed by lumbar incisions and removed completely. Rats were subsequently prepared for coronary artery ligation, and more than 30 min elapsed from the time of adrenalectomy to tightening of the ligature.

6-Hydroxydopamine-induced sympathectomy 6-Hydroxydopamine ($50\ mg\ kg^{-1}$) was dissolved immediately prior to injection in saline containing ascorbic acid (10% w/v) and administered via a tail vein to ether-anaesthetized chronically adrenal medullated rats. Coronary artery ligation was performed 6 h later. This protocol was chosen because this dose of 6-hydroxydopamine administered intravenously maximally depletes myocardial catecholamine content at this time (Kostrzew & Jacobowitz, 1974), but problems of supersensitivity of adrenoceptors would not have arisen.

Myocardial glycogen levels To assess whether 6-hydroxydopamine mimicked the putative protective effect of reserpine on myocardial glycogen stores, 6 rats received the drug as detailed above. Six hours after injection the rats were killed, hearts were rapidly removed and perfused retrogradely. After 15 min of perfusion, hearts were freeze-clamped using Wollenberger tongs previously cooled to the temperature of liquid nitrogen. Tissue was digested in alcoholic potassium hydroxide and glycogen was then hydrolysed and assayed as glucose equivalents.

In vitro *studies*

Heart perfusion Hearts were obtained from male Wistar rats (170–340 g) and perfused retrogradely with modified Krebs-Henseleit solution (composition in mM: $CaCl_2$ 2.5, KCl 1.3, $MgSO_4$ 1.2, NaCl 118, KH_2PO_4 1.2, $NaHCO_3$ 24.9, glucose 11.5) at a constant flow of $10\ ml\ min^{-1}$. The perfusate was previous-

ly gassed with 95% O_2, 5% CO_2 and maintained at 37°C. A ligature was loosely sited around the left coronary artery at the point were the vessel emerged from under the left atrium.

Surface electrical records were obtained from thin wire electrodes placed on the right atrium and apex of left ventricle. Epicardial electrograms were monitored continuously on a Narco storage oscilloscope and recorded, as required, on a Lectromed MX 216 recorder. Developed tension was assessed at a diastolic tension of 2 g, using a Devices UF1 transducer which was attached to the apex of the heart via a fine pin and thread. Recordings of developed tension and heart rate were displayed on a Devices M19 recorder.

Protocol for coronary artery ligation in vitro All hearts were allowed a 15 min stabilization period from the onset of perfusion. Only hearts which had a stable heart rate and contractile activity at the end of the stabilization period were accepted for study. Following the stabilization period, the drug under investigation was perfused continuously, beginning 15 min before the coronary artery ligature was tightened. Ventricular arrhythmic activity was recorded for 30 min post-ligation.

VT was taken as a run of five or more PVCs. VF was taken as the appearance of chaotic electrical activity with the loss of contractile activity. Five parameters of ventricular arrhythmias were always quantified during the 30 min of ligation: number of PVCs; percentage incidence of VT; duration of VT; percentage incidence of VF and duration of VF. When the α-adrenoceptor blockers were compared for antiarrhythmic effects, local anaesthetic and α-adrenoceptor blocking potency, it was desirable to have one overall index of antiarrhythmic activity. Therefore, an arrhythmia score was devised on an arbitrary scale as follows: 1 per 100 PVCs, 1 per 10% VT, 1 per 10 s VT, 1 per 10% VF, 1 per 100 s VF.

[^{3}H]-noradrenaline overflow during coronary artery ligation Hearts were perfused for a stabilization period of 15 min and instrumented as described earlier. [^{3}H]-noradrenaline ((−)-7,8-[^{3}H]-noradrenaline, total of 17.5 μCi, 8 Ci mM^{-1}, Amersham Radiochemical Centre, diluted in physiological saline) was infused for 10 min. A 20 min washout period was allowed to remove radioactivity present in the interstitial fluid, with 1 min samples collected every 5 min. After the 20 min washout period, the perfusate was collected continuously at 1 min intervals for the remainder of the experiment, as illustrated in Figure 3a.

In experiments in which an uptake inhibitor was present in the perfusate, it was allowed a contact time of 15 min prior to ligation, as mentioned earlier for all other drugs.

Samples of perfusate (1 ml) were transferred to polyethylene mini-vials containing 4 ml of scintillation fluid (Aqua-Luma, LKB). The samples were counted in a LKB 1215 Rack β liquid scintillation counter. Quenching of the samples was estimated using the external standard channels ratio method.

Perfusate lactate concentrations One ml aliquots of perfusate were collected at intervals indicated on Figure 3b. Perfusate lactate content was assessed by the method of Bergmeyer (1965) and expressed as nmol ml^{-1} g^{-1} wet weight of ventricular myocardium.

Quantification of α-adrenoceptor blocking potency Assessment of the potency of the α-adrenoceptor blockers on the myocardium proved to be impractical due to the small effect of α-adrenoceptor stimulation on cardiac function. Therefore, their potencies were determined in rat anococcygeus muscle, which has well characterized α-adrenoceptors (Gillespie, 1972). Anococcygeus muscles from male Wistar rats were placed in a small organ bath through which Krebs-Henseleit solution was perfused. Noradrenaline was administered via an injection port positioned immediately before the organ bath. Antagonists were administered in the perfusion fluid and were allowed a 15 min contact time before the noradrenaline dose-response curves were reassessed. The method of Arunlakshana & Schild (1959) was used to calculate the pA_2 for competitive blockers, and the method of Van Rossum (1963) was used to calculate the pD'_2 for phenoxybenzamine.

Quantification of local anaesthetic potency Sciatic nerves were removed from pithed frogs, partially desheathed, and placed in an apparatus containing three chambers. The proximal end of the nerve was suspended across two platinum electrodes and stimulated with square wave pulses of 0.01 ms duration and 6 V (supramaximal) amplitude delivered by a Grass S8 stimulator. The central portion of the nerve was earthed and perfused with frog Ringer. The distal portion of the nerve was placed on a platinum electrode, which was connected to a Tektronix 502A oscilloscope. After a stabilization period, drugs were administered cumulatively with a contact time of 30 min at each concentration. The EC_{50} was taken as the concentration of drug which reduced the amplitude of the action potential to 50% of its maximum value.

Statistical analysis

All values are represented as mean ± s.e.mean or percentage incidence. χ^2 analysis was used to test the data on the incidence of VT, VF and mortality, with the inclusion of the Yates correction factor for the special case of a 2 × 2 contingency table. The non-

parametric Wilcoxon Rank Sum test was used to compare drug-treated values, with those of controls, of the number of PVCs, duration of VT and VF, and plasma catecholamine concentrations. To test for statistical significance of changes in mean arterial blood pressure, heart rate and glycogen levels Student's unpaired t test (two-tailed) was used. In all tests $P < 0.05$ was considered to be statistically significant.

Drugs used

Drugs used were: atenolol, (−)-propranolol hydrochloride, (+)-propranolol hydrochloride (ICI), desipramine hydrochloride (Geigy), phentolamine mesylate (Ciba), lignocaine hydrochloride (Astra), noradrenaline hydrogen tartrate, normetanephrine hydrochloride, quinidine hydrochloride, 6-hydroxydopamine hydrochloride (Sigma), phenoxybenzamine hydrochloride (SKF), prazosin hydrochloride, trimazosin hydrochloride (Pfizer).

With the exception of prazosin, trimazosin and phenoxybenzamine, all drugs were dissolved in physiological saline. Prazosin and trimazosin were dissolved in dimethylacetamide for all experimental procedures. Phenoxybenzamine was dissolved in dimethylacetamide for the estimation of local anaesthetic and α-adrenoceptor blocking potency, and in 70% v/v ethanol in experiments involving coronary artery ligation.

Results

In vivo *studies*

Development of ventricular arrhythmias in the anaesthetized rat Left coronary artery ligation of anaesthetized rats produced arrhythmias which were similar in incidence of VT, VF, and mortality to those found previously (Clark *et al.*, 1980; Marshall *et al.*, 1981) (Table 1). However, as it is of importance for the contrast of the *in vitro* results presented later, the onset of PVCs in our *in vivo* study was more delayed compared to that observed by other workers (Figure 1a).

Neither chronic adrenal demedullation nor acute adrenalectomy significantly affected any of the parameters of ventricular arrhythmias (Table 1). It may be argued that the adrenal demedullated group had a reduced incidence of VF and mortality. However, these differences did not attain statistical significance (χ^2 value of 0.048 and 0.099 respectively for fibrillation and mortality) and the most pertinent feature of this surgically-treated group was that fibrillation was indeed present.

Changes in heart rate (Chadda *et al.*, 1974) and arterial blood pressure (Verrier *et al.*, 1974) have been shown to influence the incidence of ventricular arrhythmias in the early phase of acute myocardial ischaemia, but fortunately neither demedullation nor adrenalectomy significantly affected these parameters (Table 2).

Concomitant chemical sympathectomy and chronic adrenal demedullation abolished the occurrence of ventricular fibrillation and significantly reduced the incidence of the other less severe arrhythmias (Table 1). However, this group had appreciable haemodynamic changes with a significant decrease in mean arterial blood pressure ($P < 0.01$) accompanied by small pulse pressures. Heart rates were reduced, although not significantly (Table 2). Biochemical changes were also noted in the myocardium of this group. Glycogen content in a control group was $15.5 \pm 1.0\,\mu$mol glucose g^{-1} wet weight which was similar to those found by other workers (Gaudel *et al.*, 1979). Chemical sympathectomy with 6-hydroxydopamine produced a large increase in myocardial glycogen content ($29.1 \pm 4.3\,\mu$ mol glucose g^{-1} wet weight, $P < 0.01$).

Plasma catecholamine concentrations during coronary artery ligation Concentrations of plasma catecholamines were measured in pentobarbitone-anaes-

Table 1 Incidence of ventricular arrhythmias during 30 min of coronary artery ligation in anaesthetized rats

	n	*PVCs*	*% VT*	*VT duration*	*% VF*	*VF duration*	*% mortality*
Control	10	633 ± 170	100	44 ± 16	60	394 ± 233	20
Adrenal demedullation	9	546 ± 196	100	29 ± 10	44	361 ± 323	11
Adrenalectomy	10	660 ± 214	100	34 ± 15	60	640 ± 274	30
Adrenal demedullation + 6-hydroxydopamine	8	173 ± 73*	75	8 ± 3*	0	—	0

PVCs = premature ventricular contractions; VT = ventricular tachycardia; VF = ventricular fibrillation. Chronic adrenal demedullation or acute adrenalectomy did not significantly affect any parameter, but the combination of adrenal demedullation with 6-hydroxydopamine treatment (50 mg kg^{-1} i.v.) was protective. Values are represented as mean ± s.e.mean and significant differences from controls are denoted $*P < 0.05$.

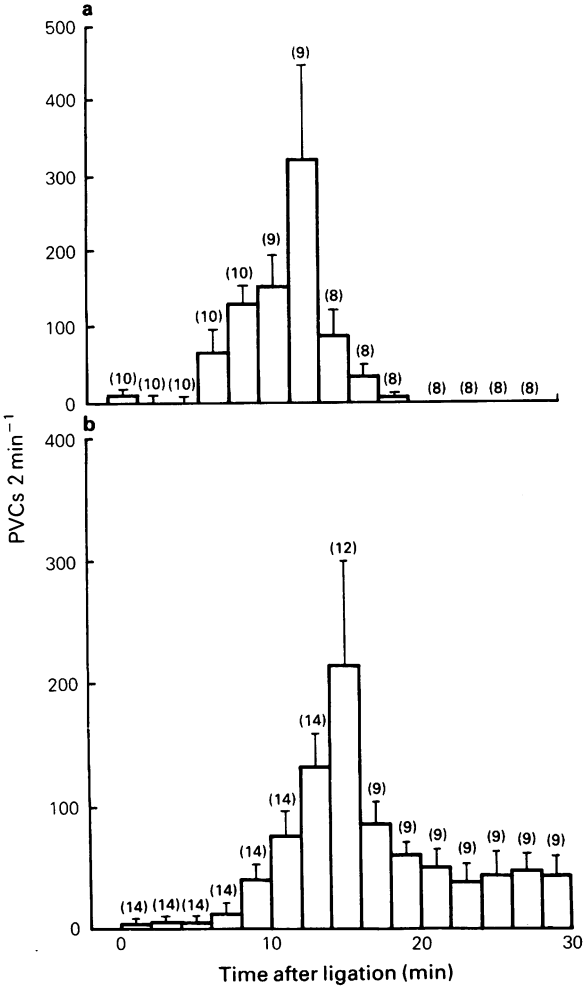

Figure 1 (a) The time course of the occurrence of premature ventricular contractions (PVCs) during the 30 min of coronary artery ligation *in vivo* in the control group of pentobarbitone-anaesthetized rats. Number of observations is represented in parentheses and decreases as rats were excluded due to death from ventricular fibrillation (VF) (b) The time course of the occurrence of PVCs during the 30 min of coronary artery ligation *in vitro* in the control group of perfused hearts. Number of observations is represented in parentheses and decrease as rats were excluded due to occurrence of VF.

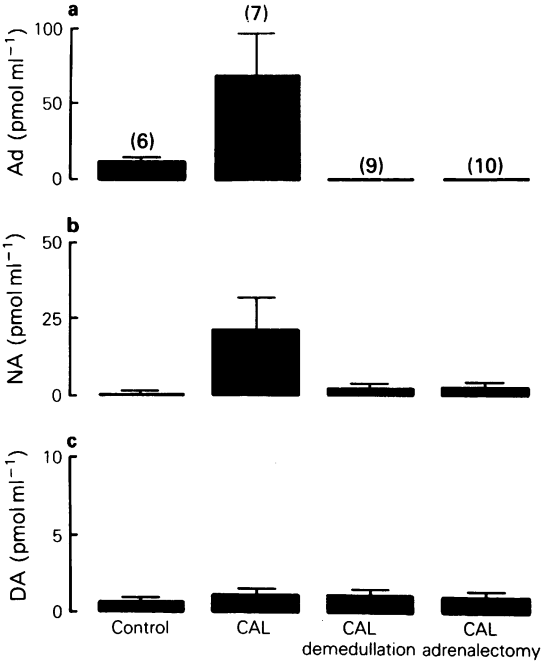

Figure 2 Plasma concentrations of (a) adrenaline (Ad), (b) noradrenaline (NA) and (c) dopamine (DA) after coronary artery ligation (CAL) in anaesthetized rats. Groups are those subjected to CAL alone and those which were either adrenal demedullated or adrenalectomized in addition to CAL. Plasma concentrations were also determined in pentobarbitone-anaesthetized animals which had not been subjected to CAL to assess basal levels (denoted as 'control'). Number of determinations is represented in parentheses.

thetized rats which were not subjected to coronary artery ligation. Plasma concentrations of adrenaline (5.1 ± 0.1 pmol ml^{-1}), noradrenaline (1.2 ± 0.3 pmol ml^{-1}) and dopamine (1.1 ± 0.4 pmol ml^{-1}) were similar to those found by other authors in the rat (Buhler *et al.*, 1978). Coronary artery ligation alone produced a large increase in plasma concentrations of adrenaline and noradrenaline, while dopamine concentrations were similar to those seen in the control group (Figure 2). In particular, the two rats which died from ventricular fibrillation in this group had very high concentrations of adrenaline and noradrenaline (232 and 139 pmol ml^{-1}; 84 and 48 pmol ml^{-1}, respectively.

In the groups of adrenal demedullated and adrenalectomized rats, plasma adrenaline concentrations were at the lower limits of detection, while noradrenaline and dopamine concentrations were not significantly different from those of the group not subjected to coronary artery ligation. Particularly pertinent was that plasma catecholamines in these surgically-treated groups were low even in rats in which persistent VF occurred.

In vitro *studies*

Occurrence of ventricular arrhythmias Fibure 1b shows the time course of the occurrence of PVCs

following coronary artery ligation in the isolated perfused heart for comparison with results obtained in the *in vivo* study. After an initial quiescent period of 8 min the incidence of PVCs increased to a peak after 12 min and then declined slightly. The prolonged runs of VT and VF also had an onset which was similar to that of the peak of PVCs. This onset of arrhythmias was similar to that seen in the pentobarbitone-anaesthetized rat, although in the anaesthetized rat there was a more dramatic reversion to an electrically stable condition (Figure 1a).

During ligation, heart rate remained unchanged while developed tension was reduced in the first 5 min and then remained constant for the rest of the 30 min of observation.

Release of metabolites during coronary artery ligation

After loading the hearts with [^{3}H]-noradrenaline, there was a gradual decrease in the amount of ^{3}H present in the perfusate over the following 20 min period, representing washout of the interstitial space. In the control series of experiments, coronary artery ligation produced a small decrease in ^{3}H overflow which stabilized after approximately 3 min, and was followed by a period in which the overflow remained similar for each time interval. Hence, no release could be detected that was temporally related to the onset or peak of arrhythmias (Figure 3a).

These experiments were repeated in the presence of an uptake 1 inhibitor, desipramine (1 μM), or an uptake 1 inhibitor, normetanephrine (3 μM), at concentrations which substantially inhibit their respective uptake 2 inhibitor, normetanephrine (3 μM), at concentrations which substantially inhibit their respective overflow was identical to that seen in the absence of drug (data not shown). A control series of experiments was performed in which hearts were loaded with [^{3}H]-noradrenaline but ischaemia was not induced. The slope for the final phase of the washout curve for this control group was the same as in groups subjected to coronary artery ligation.

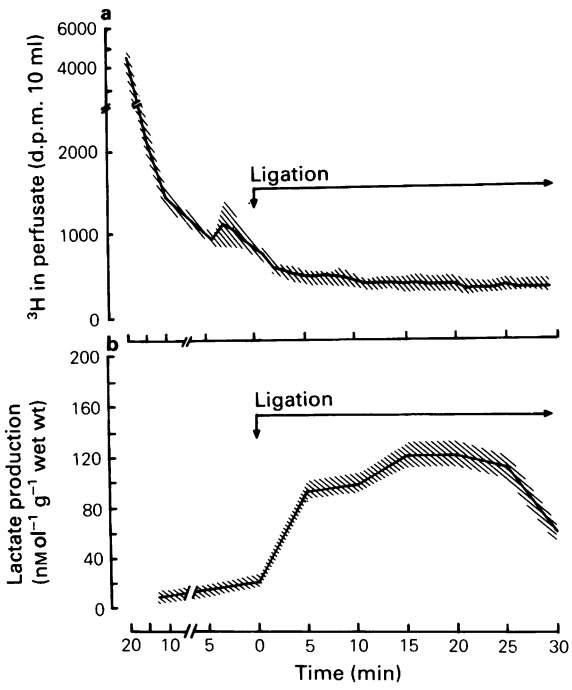

Figure 3 (a) The effects of coronary artery ligation on the release of ^{3}H from hearts preloaded with [^{3}H]-noradrenaline *in vitro*. The line represents the mean of 5 observations; s.e.mean are shown by the hatched area. (b) Effects of coronary artery ligation on lactate release from the myocardium *in vitro*. The line represents the mean of 5 observations; s.e.mean are shown by the hatched area.

Despite this inability to detect enhanced release of ^{3}H during ligation, increased concentrations of lactate were present in the effluent after occlusion, demonstrating that washout of metabolites from the ischaemic region occurred (Figure 3b).

Table 2 Heart rate and mean arterial blood pressure measured immediately before tightening the coronary artery ligature in anaesthetized rats

	n	*Heart rate (beats min^{-1})*	*Mean arterial blood pressure* (mmHg)
Control	10	375 ± 10	93 ± 7
Adrenal demedullation	9	413 ± 15	88 ± 5
Adrenalectomy	10	378 ± 7	87 ± 5
Adrenal demedullation + 6-hydroxydopamine	8	355 ± 6	68 ± 2*

Adrenal demedullation or adrenalectomy did not significantly affect either parameter, although adrenal demedullation combined with 6-hydroxydopamine administration (50 mg kg^{-1}, i.v.) reduced mean arterial blood pressure. Values are represented as mean ± s.e.mean and significant differences from controls are denoted *$P < 0.01$.

Desipramine (1 μM) was found to have very potent antiarrhythmic properties, with only a few PVCs occurring during ligation. Normetanephrine (3 μM) also possessed some minor antiarrhythmic properties as shown by a significant reduction in VF duration ($P<0.05$), although it was without effect on other ventricular arrhythmias (Table 3).

Adrenoceptor blockers on development of ventricular arrhythmias The potent β-adrenoceptor blocker, atenolol (1 μM; a concentration considerably above the pA_2 for cardiac tissue), failed to modify the development of ventricular arrhythmias. In contrast, the (−)-isomer of propranolol (1 μM) reduced the number of PVCs ($P<0.01$) and duration of VT ($P<0.01$) whilst short runs of VF were seen in only two experiments. However, the (+)-isomer of propranolol was equally effective in preventing arrhythmias (Table 3), which suggests that the antiarrhythmic effect was not due to β-adrenoceptor blockade, but rather to a non-specific effect of propranolol.

Phentolamine and prazosin (10 μM), significantly reduced the number of PVCs ($P<0.01$), the duration of VT ($P<0.01$) and the incidence of VF ($P<0.01$ for phentolamine, $P<0.05$ for prazosin). Although these two drugs were effective, two other α-adrenoceptor blockers, trimazosin (10 μM) and phenoxybenzamine (10 μM), did not significantly influence the development of arrhythmias (Table 3).

None of the drugs used, at the stated concentrations, had any significant effect on heart rate or developed tension.

Pharmacological profile of α-adrenoceptor blockers To help establish the possible mechanism of the antiarrhythmic effects of phentolamine and prazosin, their potencies as α-adrenoceptor blockers and local anaesthetics were assessed and compared with phenoxybenzamine and trimazosin.

pA_2 and pD'_2 values for blocking potency were determined on rat anococcygeus muscle using noradrenaline as the agonist. Values for the pA_2 of prazosin (9.1, $n=5$), phentolamine (8.1, $n=8$) and trimazosin (6.8, $n=6$) were in good agreement with those determined by other workers (Doxey *et al.*, 1977; Constantine & Hess, 1981). The pD'_2 value for phenoxybenzamine of 8.2 ($n=7$) was also similar to that found by Doxey *et al.* (1977) (Figure 4c).

In the absence of facilities to record cardiac action potentials, measurement of local anaesthetic potency on frog sciatic nerve was taken as an index of direct membrane effects. Although this preparation is not as sensitive as cardiac tissue to local anaesthetic properties of drugs, it was used in this study to compare the relative potency of the α-adrenoceptor blockers. EC_{50} of phentolamine was 0.65 mM. Other α-adrenoceptor blockers all required dissolution in dimethylacetamide. The volume of dimethylacetamide required to obtain sufficient concentrations of these drugs (2 ml 100 ml^{-1}) itself reduced the amplitude of the action potential by 19% which was taken into account in the determination of EC_{50}. EC_{50} of prazosin was 0.61 mM and hence had a similar local anaesthetic potency to phentolamine. Neither phenoxybenzamine ($n=7$) nor trimazosin ($n=5$) reduced the action potential sufficiently for an EC_{50} to be determined (Figure 4).

When comparing the antiarrhythmic properties with the α-adrenoceptor blocking and local anaesthetic potencies of phentolamine, prazosin, phenoxy-

Table 3 Effect of catecholamine uptake inhibitors and adrenoceptor blockers on early post-ligation arrhythmias *in vitro*

Treatment	*Conc.* (μM)	n	*PVCs*	*% VT*	*VT duration (s)*	*% VF*	*VF duration (s)*
Control		14	607 ± 93	86	54 ± 11	64	563 ± 134
Desipramine	1	6	20 ± 16**	0**	—	0*	—
Normetanephrine	3	6	662 ± 133	100	53 ± 10	67	65 ± 32*
Atenolol	1	10	636 ± 110	100	52 ± 9	80	541 ± 168
(−)-Propranolol	1	10	213 ± 39**	90	16 ± 4**	20	13 ± 6*
(+)-Propranolol	1	9	193 ± 44**	67	14 ± 3**	22	49 ± 52*
Phentolamine	1	10	747 ± 127	100	65 ± 10	60	186 ± 45
	10	12	247 ± 50**	33	8 ± 1**	0*	—
Prazosin	1	10	525 ± 144	100	51 ± 4	50	243 ± 150
	10	9	90 ± 23**	67	6 ± 2**	11*	5
Trimazosin	10	10	748 ± 219	90	51 ± 13	30	740 ± 337
†Dimethylacetamide		10	697 ± 79	100	53 ± 9	70	333 ± 146
Phenoxybenzamine	10	11	710 ± 167	55	47 ± 18	27	610 ± 113
‡Ethanol		10	628 ± 203	100	54 ± 17	60	505 ± 212

Values are represented as mean ± s.e.mean and significant differences are denoted *$P<0.05$, **$P<0.01$. †Solvent for prazosin and trimazosin; ‡solvent for phenoxybenzamine.

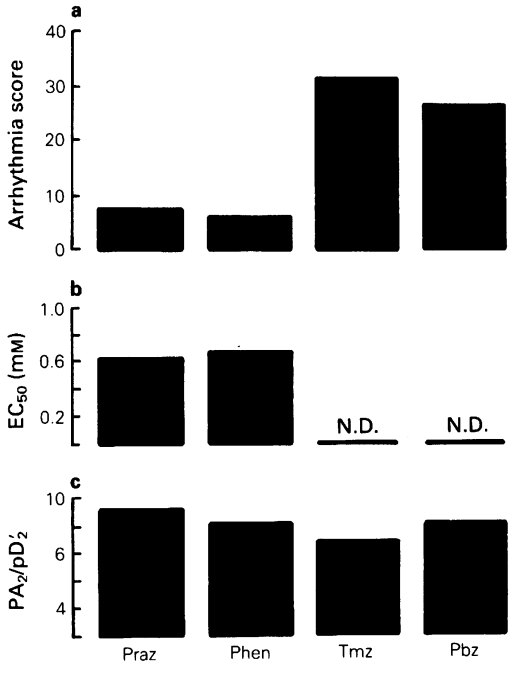

Figure 4 Effects of α-adrenoceptor blockers on: (a) Antiarrhythmic action represented as an arrhythmia score in the presence of 10 μM of the drug. The arrhythmia score is explained in the Methods section. (b) Concentration of drug which reduced the amplitude of the frog sciatic nerve action potential to 50% of its maximal value. (N.D. – local anaesthetic effect was not detectable). (c) α-Adrenoceptor blocking potency using pA_2 and pD'_2 as determinants. Praz = prazosin; Phen = phentolamine; Tmz = trimazosin; Pbz = phenoxybenzamine.

benzamine and trimazosin, it was necessary to have one index of ventricular arrhythmias from the five parameters recorded. This scale was devised only for graphical representation and would have produced a similar pattern regardless of the criteria. It was determined in the presence of 10 μM of the α-adrenoceptor blockers; a concentration which was considerably higher than the pA_2 for all of the drugs. Values calculated were: phentolamine, 6.5, prazosin, 9.3, phenoxybenzamine, 26.1, and trimazosin, 31.8. Arrhythmia score in the absence of any drug was 32.0. When comparing pharmacological properties of these drugs (Figure 4), there was no relationship between antiarrhythmic properties and α-adrenoceptor blocking potency. However, comparison between local anaesthetic potency and arrhythmia score was more striking. Phenoxybenzamine and trimazosin were found to have little antiarrhythmic activity and no detectable local anaesthetic activity. In contrast, prazosin and phentolamine were approximately equipotent for local anaesthetic and antiarrhythmic effects.

Local anaesthesia on development of ventricular arrhythmias Quinidine and lignocaine were examined to test whether antiarrhythmic drugs with local anaesthetic properties were effective in this paradigm of ventricular arrhythmias, and to establish whether this property of the α-adrenoceptor blockers could have conferred the protection seen with these drugs.

At the lower concentration of quinidine used (1 μM), only the duration of VF was significantly reduced ($P<0.01$) while a higher concentration (10 μM), reduced the number of PVCs ($P<0.01$) and incidence of VT ($P<0.01$) and abolished the incidence of VF. Lignocaine was less effective in preventing arrhythmias, with 1 μM having no significant effects, although 10 μM reduced the incidence of VT ($P<0.01$) and VF ($P<0.05$) (Table 4). Neither drug significantly affected cardiac function at the concentrations used.

Discussion

In vivo *studies*

This study has shown that the production of ventricular arrhythmias during the early phase of acute myocardial ischaemia in pentobarbitone-anaesth-

Table 4 Effects of lignocaine and quinidine on early post-ligation ventricular arrhythmias *in vitro*

Treatment	*Conc.* (μM)	n	*PVCs*	*% VT*	*VT duration (s)*	*% VF*	*VF duration (s)*
Control		14	607 ± 93	86	54 ± 11	64	563 ± 134
Lignocaine	1	6	522 ± 166	83	30 ± 8	50	347 ± 56
	10	9	330 ± 116	22**	19 ± 5	11*	9
Quinidine	1	7	555 ± 122	100	35 ± 7	42	34 ± 6**
	10	9	230 ± 75*	22**	10 ± 8	0**	—

Values are represented as mean ± s.e.mean and significant differences from control values are denoted *$P<0.05$, **$P<0.01$.

etized rats was not dependent upon elevated concentrations of plasma catecholamines.

A direct relationship between elevated plasma catecholamine concentrations and those ventricular arrhythmias occurring during the early phase of coronary artery ligation has been suggested (Ceremuzynski *et al.*, 1969). Plasma catecholamine (primarily adrenaline) concentrations in anaesthetized dogs were increased during acute myocardial ischaemia, and a relationship was found between blood catecholamine concentrations and the severity of ventricular arrhythmias (Ceremuzynski *et al.*, 1969). The present study also demonstrated high plasma concentrations of adrenaline and noradrenaline in rats subjected to coronary artery ligation alone. Very high plasma concentrations of adrenaline and noradrenaline were observed in two control animals, in this series of experiments, which died from ventricular fibrillation. These findings are in good agreement with the data of Ceremuzynski *et al.* (1969). These workers suggested that elevated plasma catecholamine concentrations caused arrhythmias, but the results produced in the present study allow an alternative interpretation. The fact that acute adrenalectomy or chronic adrenal demedullation had no significant effect on the development of ventricular arrhythmias, while dramatically reducing the circulating plasma adrenaline concentrations, was indicative that adrenaline released in the control group was probably caused reflexly; either in response to the rapid haemodynamic fluctuations caused by the arrhythmias, or as part of a general physiological response to myocardial ischaemia with no obvious detrimental effects on development of arrhythmias.

The findings that adrenal demedullation or adrenalectomy abolished increased concentrations of plasma catecholamines during coronary artery ligation, confirmed the observations of other workers (Kelliher *et al.*, 1975), who implicated the adrenal medullae as the source of elevated plasma catecholamine concentrations observed during the initial stages of myocardial ischaemia.

Lack of a significant effect of acute adrenalectomy on the development of ventricular arrhythmias, while greatly reducing plasma adrenaline concentrations, complements the findings in the chronically adrenal demedullated animals. The absence of a beneficial effect of acute adrenalectomy in the present study is contrary to the protective effect of adrenal vein ligation observed in the cat (Kelliher *et al.*, 1975). The inconsistency may also be attributable to the α-chloralose used to anaesthetize the cats compared to pentobarbitone used in the rat. However, the apparent difference may be a consequence of statistical analysis. Our reassessment of the data of Kelliher *et al.* (1975) using χ^2 analysis with the Yates correction factor yields a χ^2 value of 2.272, which would not be considered statistically significant. Therefore, the apparent protection of adrenal vein ligation on the production of ventricular arrhythmias observed in the cat may not have been a real effect.

Combination of chronic adrenal demedullation and chemical sympathectomy greatly reduced the severity of ventricular arrhythmias following coronary artery ligation. A superficial explanation of this protection may be that depletion of catecholamines from the sympathetic nerve terminals reduced the release of noradrenaline within the ischaemic myocardium. However, it is clear that other factors must be taken into account. Reserpine-induced catecholamine depletion is known to produce morphological and biochemical changes which may subsequently lead to protective effects which would not be observed with catecholamine depletion *per se*. Protective effects of reserpine-induced catecholamine depletion on the ischaemic myocardium have been attributed to the enhanced glycogen content of myocardium (Scheuer & Stezoski, 1970; Gaudel *et al.*, 1979), which may have beneficial electrophysiological consequences (Bricknell & Opie, 1978). In accord with the effects of reserpine, 6-hydroxydopamine produced a large increase in myocardial glycogen content. The presence of an increased myocardial content of glycogen may have reduced the ischaemia-induced action potential duration shortening (Cowan & Vaughan Williams, 1980). In addition to these metabolic effects, interpretation of the 6-hydroxydopamine-induced reduction in ventricular arrhythmias *in vivo* must also take account of the different haemodynamics in this group of animals.

Although the results obtained from our *in vivo* study provide evidence that plasma catecholamines are not necessary mediators of the production of arrhythmias following coronary artery ligation, they do not exclude the possibility that catecholamines released locally within ischaemic myocardium be important arrhythmogenic mediators. Hence coronary artery ligation in the isolated perfused rat heart was used to investigate this possibility.

In vitro *studies*

Use of isolated perfused rat hearts for the investigation of ventricular arrhythmias The use of an *in vitro* paradigm of coronary artery ligation to investigate ventricular arrhythmias assumed that the arrhythmias produced *in vivo* arose from factors inherent to the myocardium. In support of this assumption, there was a close similarity in the onset of ventricular arrhythmias in the present *in vitro* study compared to that observed *in vivo*, possibly indicative that the arrhythmias are initiated by mechanisms within the myocardium. Although the onset of arrhythmias was very similar in both models, following the peak of ven-

tricular arrhythmic activity *in vitro*, hearts did not revert to a stable condition as rapidly as *in vivo*. The reason for this difference is unknown, but an extracardiac influence would be implicated from these results.

Since the present study attempted to clarify the involvement of the adrenergic nervous system in the production of ventricular arrhythmias, it was necessary to postulate that the proposed involvement of adrenergic mechanisms in the production of ventricular arrhythmias was due to intramyocardial release of catecholamines from neuronal stores due to factors within ischaemic myocardium such as hyperkalemia and acidosis. This was considered as a reasonable postulate for this *in vitro* study because, although enhanced sympathetic outflow has been demonstrated during the early phase of conronary artery ligation *in vivo* (Gillis, 1971), both acute sympathetic denervation in the dog (Ebert *et al.*, 1970) and ganglion blockade with chlorisondamine in the rat (Abrahamsson *et al.*, 1982) do not influence the incidence of ischaemia-induced ventricular arrhythmias. Both of these procedures remove effects of extrinsic neural activity during ischaemia, but were ineffective at altering electrical stability of the myocardium. Both procedures would not affect the possible release of catecholamines due to local conditions within ischaemic myocardium.

Release of catecholamines from ischaemic myocardium

To investigate release of noradrenaline from ischaemic myocardium, we employed the widely used method of prelabelling neuronal pools with [^{3}H]-noradrenaline. This technique labels noradrenaline pools which are readily releasable by nerve stimulation and indirectly acting sympathomimetics. It is probable that this readily releasable pool would be the most susceptible to release by ischaemia, if this phenomenon occurred. The specific radioactivity of the tritiated noradrenaline used was very high (8 Ci mM^{-1}) and consequently only a small enhancement of release would have been detectable. The present study failed to show that ischaemia produced an enhancement of ^{3}H release from perfused hearts which were preloaded with [^{3}H]-noradrenaline. This lack of enhanced overflow occurred even though a considerable amount of releasable [^{3}H]-noradrenaline was present during the ischaemic period, as demonstrated during reperfusion studies which used a similar protocol to that of the present study (Rochette *et al.*, 1980). Besides the interpretation that catecholamines are not released during ischaemia, it may be hypothesized that any liberated noradrenaline would not have been detected due to a combination of the low flow from the ischaemic myocardium and the involvement of catecholamine uptake processes, although this is considered unlikely for reasons discussed below.

In the model used in the present study there was a residual flow through at least part of the ischaemic region to facilitate washout of noradrenaline if release had occurred, as demonstrated by the presence of lactate in the effluent. Even in the presence of desipramine (uptake 1 inhibitor) or normetanephrine (uptake 2 inhibitor) at concentrations producing marked inhibition of uptake processes (Iversen, 1965), there was no demonstrable enhanced release of ^{3}H during ischaemia. Therefore, the lack of detectable ^{3}H release during ischaemia, in the presence of inhibitors of the catecholamine uptake process and where other metabolites produced by the ischaemic myocardium are readily demonstrable, does not support the speculation that the local conditions of ischaemia produce noradrenaline release within the myocardium.

Although there is agreement on the lack of demonstrable intramyocardial noradrenaline release during the early phase of ischaemia from *in vitro* studies (Rochette *et al.*, 1980), this consistency has not been found by other workers during coronary artery ligation studies performed *in vivo*. Earlier studies in anaesthetized open-chested dogs demonstrated release of catecholamines after 2 min (Shahab *et al.*, 1972), 15 min (Dutta & Booker, 1970) and 1 h (Lammerant *et al.*, 1966) of coronary artery ligation. Recent studies in dogs, using more precise methods for determination of catecholamines have uniformly failed to detect elevated concentrations of noradrenaline in the local coronary vein from the ischaemic myocardium after 10 min (McGrath *et al.*, 1981), 15 min (Riemersma *et al.*, 1981) of coronary occlusion, although all described increased plasma concentrations of lactate which would have leaked from the ischaemic zone.

Prior to the present *in vitro* study, only two previous investigations which were both *in vivo*, have examined directly the relationship between possible intramyocardial noradrenaline release and the production of ventricular arrhythmias in the early phase of coronary artery ligation and again the results are conflicting. Hirche *et al.* (1980) found a release of noradrenaline into a local coronary vein of an ischaemic region after 3 min in the anaesthetized pig which coincided with the occurrence of the first phase of ventricular arrhythmias. However, in agreement with the present study, Marshall & Parratt (1980) failed to demonstrate such a relationship in the anaesthetized dog. The reason for these contradictory data is not readily apparent, but demonstrates that there is considerable doubt whether noradrenaline is released from the ischaemic myocardium, contributing to the production of ventricular arrhythmias.

During experiments to determine ^{3}H overflow, antiarrhythmic effects of both desipramine and normetanephrine were noted (Table 3). The effects were particularly profound in the case of desipramine,

which was the most potent of the pharmacological agents used in this study. The mechanism by which desipramine exerted its effect is not clear, but is likely to be due to its direct electrophysiological effects (Tamargo *et al.*, 1979).

Adrenoceptor blockers and development of ventricular arrhythmias The present *in vivo* study also investigated the possibility of intramyocardial release of catecholamines on ventricular arrhythmia development using pharmacological tools which block adrenoceptors.

The first site of investigation was that of β-adrenoceptors. Atenolol was used as it is a potent β-adrenoceptor blocker which possesses minimal local anaesthetic properties. The lack of any antiarrhythmic effects of atenolol suggests that β-adrenoceptors were not involved in the genesis of these ventricular arrhythmias in this model. Administration of (−)-propranolol did produce antiarrhythmic effects. However, the equipotency of the (+)-isomer in suppressing arrhythmias confuted the involvement of β-adrenoceptors, since the (+)-isomer of propranolol is approximately a hundred times less potent as a β-adrenoceptor blocker than the (−)-isomer, although both isomers possess comparable local anaesthetic potency (Barrett & Cullum, 1968).

Several studies have demonstrated antiarrhythmic properties of propranolol (Reviewed by Fitzgerald, 1982), but few have attempted to define the mechanism of this effect. Of the few mechanistic studies, Reynolds *et al.* (1978) ascribed the protective influence of propranolol to its effect in reducing heart rate, while in agreement with the present study, Fearon (1967) attributed the antiarrhythmic efficacy to non-specific effects. Fewer studies have used atenolol and results have been conflicting. Antiarrhythmic properties have been observed in the dog (Abendroth *et al.*, 1977), although no significant effects were observed in the rat (Harris *et al.*, 1982).

The other site of investigation was α-adrenoceptors, which have an undetermined physiological function in the myocardium. Despite this undetermined physiological role, α-adrenoceptor blockers produce antiarrhythmic effects during coronary artery ligation in cats (Sheridan *et al.*, 1980) and rats (Parratt *et al.*, 1981). In agreement with these two findings, in the present study antiarrhythmic effects of phentolamine and prazosin were observed in isolated perfused hearts. We sought to establish whether this antiarrhythmic effect was an α-adrenoceptor-mediated effect or related to ancillary properties of these drugs. It has been suggested previously that non-specific effects of these drugs account for their antiarrhythmic action during reperfusion (Thandroyen *et al.*, 1983; Bralet *et al.*, 1985). Rosen *et al.* (1971) showed that phentolamine had direct quinidine-like membrane depressant properties at concentrations which were required to produce significant antiarrhythmic effects in the present study. A similar effect has been demonstrated for prazosin (Northover, 1983). The present study also used two other α-adrenoceptor antagonists, phenoxybenzamine and trimazosin (a quinazoline related to prazosin; Constantine & Hess, 1981), neither of which produced any significant antiarrhythmic effects. In an attempt to clarify the effects of the four α-adrenoceptor blockers used, their relative potencies as α-adrenoceptor blockers and as local anaesthetics were determined and compared to their antiarrhythmic effects. A comparison of the pharmacological profile of the four α-adrenoceptor blockers used in the present study revealed no relationship between α-adrenoceptor blocking potency and antiarrhythmic effects. It is of interest that both prazosin and phentolamine had measurable and similar local anaesthetic potency and comparable antiarrhythmic effects, while phenoxybenzamine and trimazosin had neither discernible local anaesthetic properties nor significant antiarrhythmic efficacy. The relationship between the local anaesthetic potency and antiarrhythmic effects may be a causal phenomenon as the local anaesthesics, quinidine and lignocaine, produced antiarrhythmic activity in this model (Table 4). Further electrophysiological data are required to confirm the speculation presented in this study that the antiarrhythmic effects of prazosin and phentolamine were due to membrane depressant properties.

In summary, coronary artery ligation on the anaesthetized rat *in vivo* produced ventricular arrhythmias which were not modified by reduction of plasma catecholamine concentrations using chronic adrenal demedullation or acute adrenalectomy. Concomitant chemical sympathectomy and chronic adrenal demedullation reduced the incidence of arrhythmias, but the accompanying biochemical and haemodynamic changes associated with this treatment make it difficult to define the mechanism of this effect. Several approaches were designed *in vitro* to study the possible role of intramyocardial release of catecholamines in the production of early arrhythmias in regionally ischaemic isolated hearts of the rat. Many of the pharmacologically-induced effects studied on arrhythmias were consistent with results from other studies (both *in vivo* and *in vitro*), and we have extended these findings to determine the mechanism of action. While some inhibitors of the adrenergic system possessed antiarrhythmic activity, it was concluded that these protective effects were attributable to non-specific effects. Thus, the overall consensus using these different experimental designs was that adrenergic mechanisms are not necessary mediators of the production of ventricular arrhythmias in the early phase of ischaemia in the rat.

Alan Daugherty was supported by a British Heart Foundation Research Fellowship. The authors thank Michelle Fox for her technical assistance. The following are gratefully acknowledged for their gifts of drugs: ICI (atenolol and optical isomers of propranolol), Pfizer (prazosin and trimazosin). We thank Dr Peter B. Corr for his help and constructive criticism in the preparation of this manuscript.

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(Received June 25, 1985.
Revised August 29, 1985.
Accepted September 10, 1985.)