Br. J. Pharmacol. (1991), **102**, 533–539

Chemosensitive cardiopulmonary afferents and the haemodynamic response to simulated haemorrhage in conscious rabbits

[1]Roger G. Evans & John Ludbrook

Cardiovascular Research Laboratory, University of Melbourne Department of Surgery, Royal Melbourne Hospital, Parkville, Victoria, Australia 3050

1 We set out to test whether the signal from the heart that initiates the decompensatory phase of acute central hypovolaemia in conscious rabbits is conveyed by chemosensitive afferents.

2 Haemorrhage was simulated by inflating an inferior vena caval cuff so that cardiac output fell at a constant rate of 8% of its baseline level per min. After sham or vehicle treatments the haemodynamic response had two phases. In the first, sympathoexcitatory, phase systemic vascular conductance fell in proportion to cardiac output so that mean arterial pressure fell by only 13 mmHg. When cardiac output had fallen by ~50% a second, sympathoinhibitory, phase supervened. There was an abrupt rise of systemic vascular conductance and an abrupt fall of mean arterial pressure, to ~40 mmHg.

3 The sympathoinhibitory phase was prevented by injection of the δ-opioid antagonist ICI 174864 (100–300 nmol) or the μ-opioid agonist H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO) (100–300 pmol) into the fourth cerebral ventricle.

4 5-HT_3 receptors on myocardial or pulmonary afferents were excited by injection of ascending doses of phenylbiguanide (6.25–400 μg) into the left or right atrium respectively. Neuronal-type nicotinic cholinoceptors in the epicardium were excited by injecting ascending doses of nicotine bitartrate (6.25–400 μg) into the pericardial sac. Each of these treatment regimens caused a reproducible, dose-dependent, fall in mean arterial pressure. Intravenous injection of the 5-HT_3 antagonist MDL 72222 (1.0 mg kg^{-1}) markedly attenuated the responses to phenylbiguanide. Intrapericardial injection of the neuronal-type nicotinic cholinoceptor antagonist mecamylamine HCl (0.1 mg kg^{-1}) abolished the effects of intrapericardial nicotine. Neither of these treatments affected the haemodynamic response to simulated haemorrhage.

5 Injection into the fourth ventricle of ICI 174864 (100–300 nmol) or DAMGO (100–300 pmol) had no effects on the dose-response relationships for phenylbiguanide or nicotine.

6 We conclude that the cardiac afferents responsible for initiating the sympathoinhibitory phase of simulated haemorrhage in conscious rabbits do not correspond to the populations of phenylbiguanide-sensitive cardiopulmonary afferents, nor to the population of nicotine-sensitive epicardial afferents. We also conclude that the reflex haemodynamic responses to atrial phenylbiguanide and intrapericardial nicotine do not depend on an endogenous δ-opioid receptor mechanism in the brainstem, and are not affected by exposure of the brainstem to exogeneous DAMGO.

Introduction

When phenylbiguanide is injected into the left or right artrium it excites two anatomically distinct sets of afferents that originate, respectively, in the left side of the heart and close to the pulmonary vasculature (Evans *et al.*, 1990a). The effect of phenylbiguanide is mediated by pharmacologically-specific 5-HT_3 receptors (Wright & Angus, 1988; Evans *et al.*, 1990a). The afferents run mainly, though not exclusively, in the vagus nerves. When nicotine bitartrate is injected into the pericardial sac it excites afferents that originate in the epicardium (Evans *et al.*, 1990b). These afferents are not sensitive to phenylbiguanide. The effect of nicotine is mediated by pharmacologically-specific neuronal-type nicotinic cholinoceptors. The afferents travel exclusively in the vagus nerves. The reflex hypotension resulting from excitation of these afferents is chiefly due to a fall in peripheral resistance, presumably due to sympathoinhibition (Evans *et al.*, 1990b; Evans & Ludbrook, unpublished observations).

Sympathoinhibition resulting from the excitation of cardiac afferents is also a feature of one phase of the response to acute haemorrhage, or simulated haemorrhage, in unanaesthetized rabbits. At first, peripheral resistance increases along with the fall of cardiac output, so that mean arterial pressure is maintained at a near-normal level (Ludbrook & Graham, 1984; Schadt *et al.*, 1984; Ludbrook & Rutter, 1988; Ludbrook *et al.*, 1988). The maintenance of blood pressure is associated with a progressive rise in sympathetic drive (Burke & Dorward, 1988; Morita *et al.*, 1988) and plasma noradrenaline concentration (Schadt & Gaddis, 1985), so that this first phase can be described as sympathoexcitatory. When about 30% of blood volume has been withdrawn (Schadt *et al.*, 1984; Burke & Dorward, 1988; Ludbrook & Rutter, 1988), or when cardiac output has fallen by about 50% (Evans *et al.*, 1989a, b), a second phase begins in which there is an abrupt decrease in sympathetic activity, peripheral resistance and mean arterial pressure. This sympathoinhibition is initiated by a signal from the heart (Burke & Dorward, 1988; Evans *et al.*, 1989a) and depends on activation of a δ-opioid receptor mechanism within the central nervous system (Evans *et al.*, 1989b). The sympathoinhibition of Phase 2 can also be prevented by injecting a specific μ-opioid receptor agonist into the 4th ventricle (Evans & Ludbrook, 1990).

The properties of the cardiac afferents responsible for the second, sympathoinhibitory, phase of acute hypovolaemia in rabbits are not known, but in rats there is circumstantial evidence that they may be sensitive to phenylbiguanide. Both at the onset of the hypotensive phase of haemorrhage (Victor *et al.*, 1989) and after injection of phenylbiguanide (Higuchi *et al.*, 1988) there is a sharp decline of renal sympathetic nerve activity but an increase of adrenal sympathetic nerve activity. As is the case in rabbits, an endogenous opioid mechanism appears to mediate the severe hypotension resulting from haemorrhage in rats (Faden & Holaday, 1979).

[1] Author for correspondence.

We have set out to determine whether one or more of the populations of chemosensitive afferents described above might be responsible for the sympathoinhibitory phase of hypovolaemia, by testing the effects of a δ-opioid receptor antagonist and a μ-opioid receptor agonist on the chemoreflexes. In addition it is unclear whether 5-hydroxytryptaminergic or cholinergic mechanisms are essential to the putative physiological function of these chemosensitive cardiopulmonary afferents. We therefore tested the effects of blockade of 5-HT_3 receptors and of epicardial neuronal-type nicotinic cholinoceptors on the haemodynamic response to simulated haemorrhage.

Methods

Six New Zealand White rabbits were used, weighing 2.40–2.71 kg (mean 2.57). Each rabbit was studied 4–6 times, at intervals of 2–5 days. The experiment was conducted in accordance with the Statement on Animal Experimentation by the National Health and Medical Research Council of Australia, and was approved by the Animal Ethics Committee of the Amalgamated Melbourne and Essendon Hospitals.

Surgical preparation

The methods used to prepare the rabbits have been described in detail previously (Evans *et al.*, 1989a,b; 1990a; Evans & Ludbrook 1990). In brief, they were as follows.

The rabbits were anaesthetized with a halothane/oxygen mixture after induction with i.v. thiopentone sodium (25 mg kg^{-1}) (Pentothal, Abbott) and endotracheal intubation. Buprenorphine HCl (60 μg s.c.) (Reckitt & Colman) was administered post-operatively as an analgesic. Each rabbit underwent three separate operations. During the first operation an inflatable cuff was placed round the thoracic inferior vena cava (caval cuff). Two to three weeks later an electromagnetic flow probe (Biotronex BL5050) was placed round the ascending aorta and an inflatable cuff round the descending thoracic aorta (aortic cuff). At the same operation a catheter was either placed in the pericardial sac (3 rabbits), or inserted into the left atrium through the tip of its appendage (3 rabbits). Two weeks later a catheter was introduced through the atlanto-occipital ligament so that its tip lay in the 4th ventricle. The first study was done 10 days later.

Preparations for studies

These were done under local analgesia with 2% procaine HCl (David Bull Laboratories). The rabbit was placed in a 15 × 40 cm box fitted with a wire mesh lid, 60 min before the beginning of the study. The ends of the catheters, the tubes leading to the cuffs, and connecting plug for the flow probe were retrieved from their subcutaneous positions. A catheter was introduced into the central ear artery and advanced to the root of the ear to measure arterial pressure. A catheter was also introduced into a marginal ear vein and advanced to the right atrium for drug administration. In some studies, a catheter was introduced into the contralateral ear artery to collect arterial blood samples.

Measurement of haemodynamic and respiratory variables

The ear artery catheter was connected to a Statham P23Dc strain gauge zeroed 50 mm above the floor of the rabbit's box. The flow probe was connected to a Biotronex BL-613 meter to measure ascending aortic flow (cardiac output). Heart rate was measured by a tachometer actuated by the flow pulse. A mercury-in-rubber strain gauge was placed around the thorax and connected to a bridge-circuit (Parks Electronics Laboratory) to monitor respiration and other movements. Signals were amplified and recorded on a Grass Model 7 Polygraph.

The output from the Grass Polygraph was sent to an Olivetti M24 computer with an analogue-to-digital converter which provided 1 or 10 s mean values of arterial pressure (MAP, mmHg), cardiac output (MCO, ml min^{-1}) and heart rate (MHR, beats min^{-1}). The computer also calculated mean values for cardiac index (MCI = MCO/body weight in kg) and systemic vascular conductance index (MSVCI = 10^2 MCI/MAP).

Analysis of blood samples

Duplicate measurements of haematocrit (Hawksley) were made before each day's study on 0.2 ml samples of ear vein blood. The haematocrit at the beginning of the first and last studies was 35.8 ± 1.2% and 34.0 ± 0.8% respectively. Po_2 and Pco_2 were measured on 0.6 ml samples of ear artery blood (Radiometer ABL_4 acid-base analyser).

Simulated haemorrhage

The caval cuff was inflated gradually by means of a micrometer syringe so that MCI fell at a constant rate of 13.32 ± 0.45 ml kg^{-1} min^{-1} per min (8.29 ± 0.15% of the baseline level per min). The cuff was released when MAP had fallen to ~40 mmHg or when MCI had fallen to 39.7 ± 1.5% of its resting level.

Baroreceptor-heart rate reflex

This was elicited by inflating the aortic and caval cuffs so that MAP rose or fell at 1–2 mmHg s^{-1} (Ludbrook, 1984).

Drugs

Unless otherwise indicated, these were made up in 154 mM NaCl (saline) on the day of a study. All doses were calculated as the salt. Sham treatments consisted of the equivalent volume of vehicle.

Cardiopulmonary chemoreceptor excitants Nicotine bitartrate (TCI) and 1-phenylbiguanide (Aldrich) were administered as successive bolus doses of 6.25, 25, 100 and 400 μg in 200 μl. Phenylbiguanide was injected into the left or right atrium at 10 min intervals. Nicotine was injected into the pericardial sac at 30 min intervals. These doses cover the full range of the dose-response relationship (Evans *et al.*, 1990a,b).

Cardiopulmonary chemoreceptor antagonists These were the 5-HT_3 receptor antagonist MDL 72222 (1αH, 3α, 5αH-tropan-3yl-3,5-dichlorobenzoate) (Fozard, 1984a, b) (Research Biochemicals Inc.) and the neuronal-type nicotinic cholinoceptor antagonist mecamylamine HCl (Van Rossum, 1962) (Sigma). MDL 72222 (1.0 mg kg^{-1}) was injected i.v. in a volume of ~2.5 ml over 1 min followed by an infusion at 0.05 mg kg^{-1} min^{-1}. Mecamylamine (0.1 mg kg^{-1}) was given intrapericardially in a volume of 200 μl, repeated at 60 min intervals. We have shown that these regimens shift the dose-response curves for phenylbiguanide and nicotine approximately 16 fold to the right (Evans *et al.*, 1990a, b).

Intracerebroventricular drug administration The μ-agonist H-Try-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO) (Handa *et al.*, 1981) (Sigma) was made up as 300 pmol per 15 μl in saline. The δ-antagonist ICI 174864 (N,N-diallyl-Tyr-(α-aminoisobutyric acid)$_2$-Phe-Leu-Oh) (Cotton *et al.*, 1984) (Cambridge Research Biochemicals) was made up as 50 nmol per 15 μl of alkalinized saline (0.1 M NaOH in 154 mM NaCl). Immediately prior to 4th ventricular administration this solution was brought to pH 7–8 by addition of 1.0 M HCl. The doses of DAMGO and ICI 174864 were respectively 100 or 300 pmol and 100, 200 or 300 nmol, administered at 30 min intervals.

Experimental protocols

First, we set out to test whether 4th ventricular administration of the δ-antagonist ICI 174864 or the μ-agonist DAMGO, in doses that prevented the occurrence of Phase 2 of simulated haemorrhage, would also attenuate the hypotensive responses to ascending doses of the chemoreflex excitants phenylbiguanide (left and right atrial) and nicotine (intrapericardial). Secondly, we tested the effects on the haemodynamic response to simulated haemorrhage of the 5-HT_3 antagonist MDL 72222 (i.v.) and the neuronal-type nicotinic cholinoceptor antagonist mecamylamine (intrapericardial), in doses that markedly antagonized the corresponding chemoreflexes.

The steps in each study followed were similar. (1) Vehicle was injected by the appropriate route, then a simulated haemorrhage was performed. (2) Thirty min later the dose-response relationship for intrapericardial nicotine, or left or right atrial phenylbiguanide was constructed. (3) Thirty min later the active treatment was given, and simulated haemorrhage was repeated. (4) Thirty min later, the active treatment was given by the appropriate route and the chemoreflex dose-response relationship was again constructed. (5) When the active treatment was 4th ventricular ICI 174864 or DAMGO, it was repeated 30 min later and a further simulated haemorrhage was performed.

Step (3) was sometimes varied in the case of 4th ventricular ICI 174864 and DAMGO, since it was necessary to establish a dose that prevented Phase 2 of simulated haemorrhage. If the first dose did not do so, the higher dose was tested after a 30 min recovery period.

When the active treatment was 4th ventricular DAMGO, arterial blood gases were measured before, and at 30 min intervals during steps 3–5 to test for respiratory depression.

When the active treatment was intrapericardial mecamylamine, the baroreceptor-heart rate reflex was elicited before and after Step 3, to test for cardiac vagal ganglionic blockade.

Order of studies

In the 3 rabbits with pericardial catheters the effects of treatment with intrapericardial mecamylamine, 4th ventricular ICI 174864 and 4th ventricular DAMGO on simulated haemorrhage and the dose-response relationship for intrapericardial nicotine were tested in randomized order (3 × 3 Latin square). Then a fourth study was done, in which none of these treatments were given, to serve as a time control.

In the 3 rabbits with left atrial catheters the effects of treatment with i.v. MDL 72222 and 4th ventricular ICI 174864 and DAMGO on simulated haemorrhage, and the dose-response relationship for left atrial phenylbiguanide were tested in randomized order. Then a fourth, time control, study was done in which none of the treatments were given.

In 2 of the rabbits with pericardial catheters and 1 with a left atrial catheter the effects of treatment with 4th ventricular ICI 174864 and DAMGO on simulated haemorrhage and the dose-response relationship for right atrial phenylbiguanide were tested in randomized order. The effects of MDL 72222 were not retested, nor was the time control study repeated.

Autopsy

At the conclusion of the experiments the rabbits were killed with i.v. pentobarbitone sodium (200 mg) (Nembutal, Ceva Chemicals). Autopsies were performed on all rabbits to confirm that the catheters were properly placed and that there were no signs of infection or adhesions in the 4th ventricle or pericardial sac.

Analysis of results

Data were analysed as described previously (Evans *et al.*, 1989a,b; 1990a; Evans & Ludbrook, 1990).

Two- or three-way analyses of variance (ANOVA) were used to evaluate effects of treatments (drugs or vehicles) on the resting levels of the haemodynamic and respiratory variables. If specific contrasts were made within the ANOVA the critical value of F was adjusted by the Bonferroni method.

Regression analysis confirmed that MCI fell linearly with time during the simulated haemorrhages ($r = 0.983 \pm 0.001$). As we have found previously (Evans *et al.*, 1989a), there was a near-linear relationship of MAP, MHR and MSVCI to MCI during simulated haemorrhage, up to the onset of Phase 2 when MSVCI rose abruptly. As before (Evans *et al.*, 1989a,b) we used a graphical method for analysing the effects of treatments on the responses of MSVCI, MAP and MHR to simulated haemorrhage. The relationships of MAP, MHR and MSVCI to MCI were characterized by three sets of co-ordinates: (a) Post-treatment levels, immediately before the onset of simulated haemorrhage. (b) The point at which MSVCI reached a minimum at the end of Phase 1, before rising abruptly. (c) The final observation before the caval cuff was deflated. These co-ordinates were averaged, first within and then between rabbits, so as to distinguish the effects of the different treatments. ANOVA was used to detect effects of treatments on the levels of the haemodynamic variables immediately before caval cuff deflation.

Mean log dose-response curves were constructed for the effects of intrapericardial nicotine and left and right atrial phenylbiguanide on MAP. ANOVA was used to detect effects of treatments (treatment × dose interaction).

Unless otherwise indicated, the levels of the variables are tabulated as between rabbit means ± 1 s.e.mean. The s.e.mean for grand means across rabbits, studies and treatments was calculated from the error sum of squares in ANOVA.

Results

The baseline levels of the haemodynamic variables were unaffected by the injection of vehicle by any route ($F \leqslant 1.3$; d.f. 3,

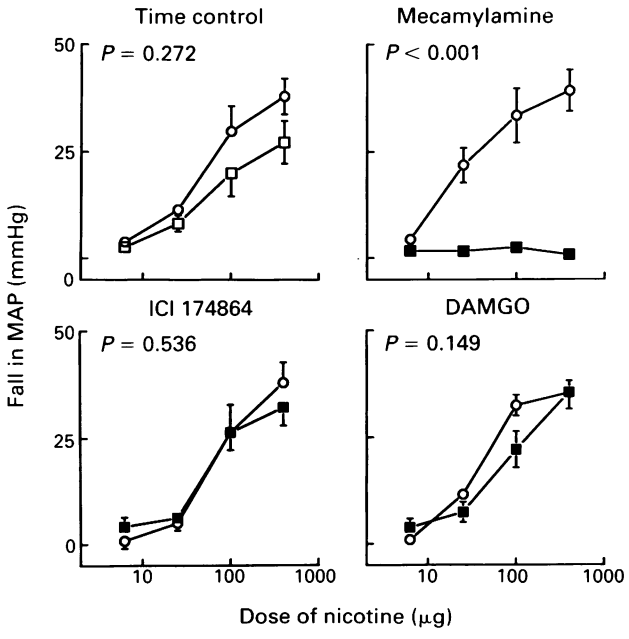

Figure 1 Dose-response relationships for intrapericardial nicotine according to the 4 studies, and treatments within studies. MAP, mean arterial pressure. Mean values for 3 rabbits. Vertical bars indicate 1 s.e.mean. Treatments are indicated by symbols: (○), no treatment (time control study) or vehicle (remaining studies), (□), no treatment (time control study), (■), intrapericardial mecamylamine (0.1 mg kg^{-1}), 4th ventricular ICI 174864 (100–300 nmol), or 4th ventricular H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO, 300 pmol). P values attributable to dose × treatment interaction within ANOVA.

14; $P \geqslant 0.33$). Their grand mean values, across rabbits and studies, were: MCI, 164 ± 9 ml kg^{-1} min^{-1}; MSVCI, $194 \pm 11\,10^2$ ml kg^{-1} min^{-1} mmHg^{-1}; MAP, 85 ± 3 mmHg; MHR, 234 ± 13 beats min^{-1}.

In the time-control studies within the three protocols the pattern of the response to simulated haemorrhage, and the dose-response relations for atrial phenylbiguanide and intrapericardial nicotine, were usually unaffected by repetition ($F \leqslant 1.4$; d.f. 3, 14; $P \geqslant 0.27$). The exception was that the rabbits became supersensitive to the effects of left atrial phenylbiguanide (Figure 3). Across studies, the patterns of the responses to simulated haemorrhage after vehicle treatment were indistinguishable (Figures 2, 4 and 6). During Phase 1 MCI fell by 80 ± 6 ml kg^{-1} min^{-1}, MSVCI by $78 \pm 5\,10^2$ ml kg^{-1} min^{-1}, and MAP by 13 ± 2 mmHg. MHR rose by 61 ± 8 beats min^{-1}. When the caval cuff was deflated at the end of Phase 2, the corresponding changes from the baseline levels were 99 ± 6 ml kg^{-1} min^{-1}, $27 \pm 8\,10^2$ ml kg^{-1} min^{-1} mmHg^{-1}, 47 ± 2 mmHg and 37 ± 9 beats min^{-1}.

Intrapericardial mecamylamine and i.v. MDL 72222 had no effects on the haemodynamic variables. In the phenylbiguanide protocols 4th ventricular ICI 174864 and DAMGO had no effect, but in the intrapericardial nicotine protocol they lowered MHR by 28 ± 14 and 36 ± 20 beats min^{-1} respectively ($F \geqslant 7.1$; d.f. 1, 6; $P \leqslant 0.04$). Fourth ventricular DAMGO, in doses that prevented Phase 2 of simulated haemorrhage (100–300 pmol), lowered respiratory rate and caused a steady fall of Po_2 over 180 min from 88 ± 2 to 75 ± 3 mmHg and a steady rise of Pco_2 from 34 ± 1 to 46 ± 2 mmHg.

Intrapericardial nicotine protocol

Intrapericardial mecamylamine (0.1 mg kg^{-1}) did not affect baseline MHR. It did reduce the range of MHR in the baroreceptor-heart rate reflex from 137 ± 7 to 102 ± 18 beats min^{-1}, suggesting that there may have been a degree of cardiac vagal ganglionic blockade, though this effect was not consistent ($F = 3.2$; d.f. 1, 2; $P = 0.27$). It completely abolished the responses of MAP to intrapericardial nicotine (Figure 1), but had no effect on the responses to simulated haemorrhage (Figure 2).

Fourth ventricular ICI 174864 (100–300 nmol) and DAMGO (300 pmol) prevented the occurrence of Phase 2 of simulated haemorrhage (Figure 2), but had no effect on the responses of MAP to intrapericardial nicotine (Figure 1).

Left atrial phenylbiguanide protocol

MDL 72222 (1.0 mg kg^{-1}) markedly attenuated the responses of MAP to left atrial phenylbiguanide (Figure 3), but had no effect on the responses to simulated haemorrhage (Figure 4).

Fourth ventricular ICI 174864 (100 nmol) and DAMGO (100–300 pmol) prevented the occurrence of Phase 2 of simu-

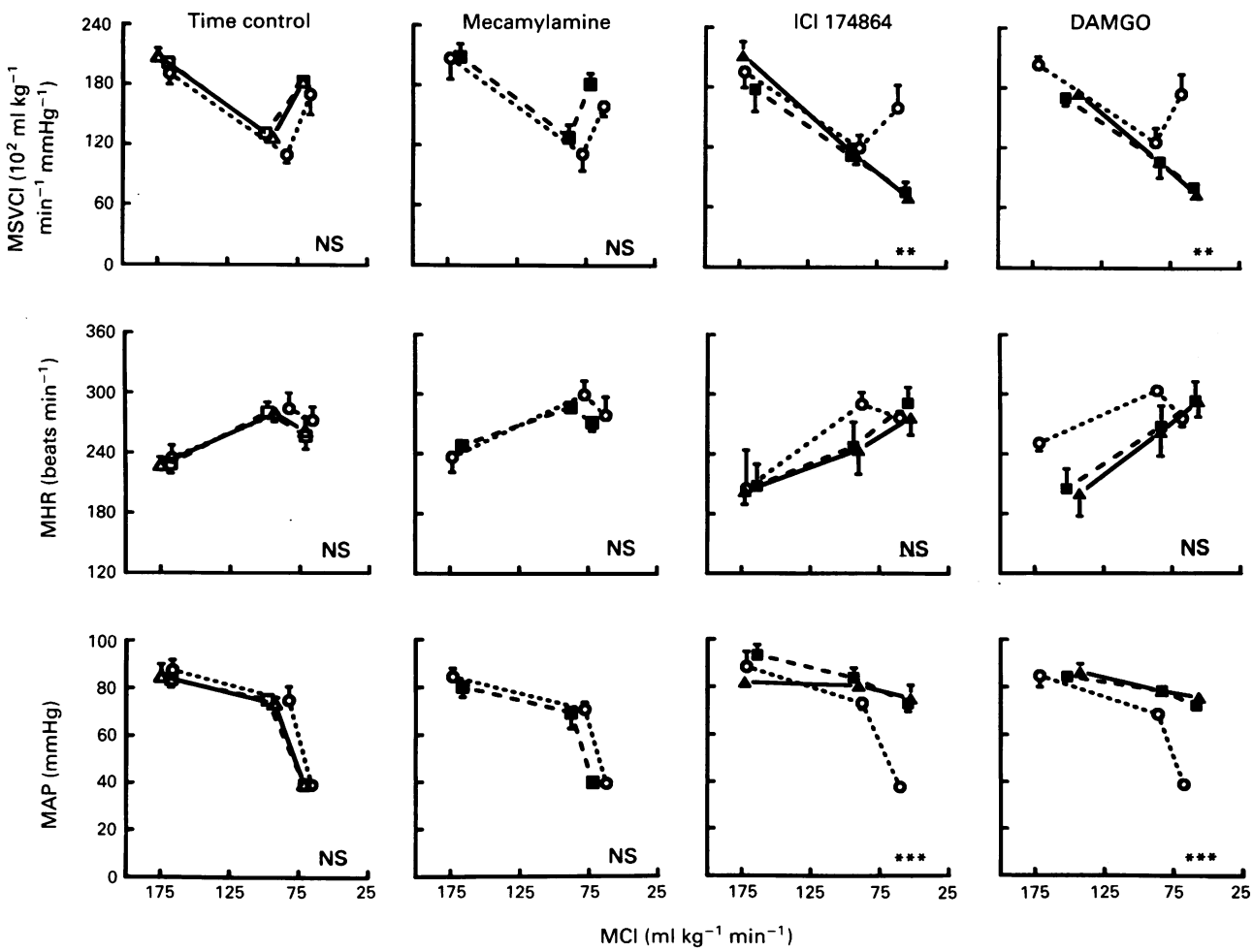

Figure 2 Haemodynamic effects of simulated haemorrhage in intrapericardial nicotine protocol according to the 4 studies, and treatments within studies. MSCVI, mean systemic vascular conductance index. MAP, mean arterial pressure. MHR, mean heart rate. MCI, mean cardiac index. Symbols indicate mean coordinates for 3 rabbits. Vertical bars indicate 1 s.e.mean. Treatments are indicated by symbols: (○) no treatment (time control study No. 1) or vehicle treatment (remaining studies); (□) no treatment (time control study No. 2); (△) no treatment (time control study No. 3); (■) intrapericardial mecamylamine (0.1 mg kg^{-1}), 4th ventricular ICI 174864 (100–300 nmol), or 4th ventricular H-Tyr-D-Ala-Gly-MePhe-NH(CH$_2$)$_2$OH (DAMGO, 300 pmol); (▲) second treatment with ICI 174864 or DAMGO. Comparisons between levels at end of simulated haemorrhage were made by ANOVA. NS, $P \geqslant 0.05$; *$0.01 \leqslant P < 0.05$; **$0.001 \leqslant P < 0.01$; ***$P < 0.001$.

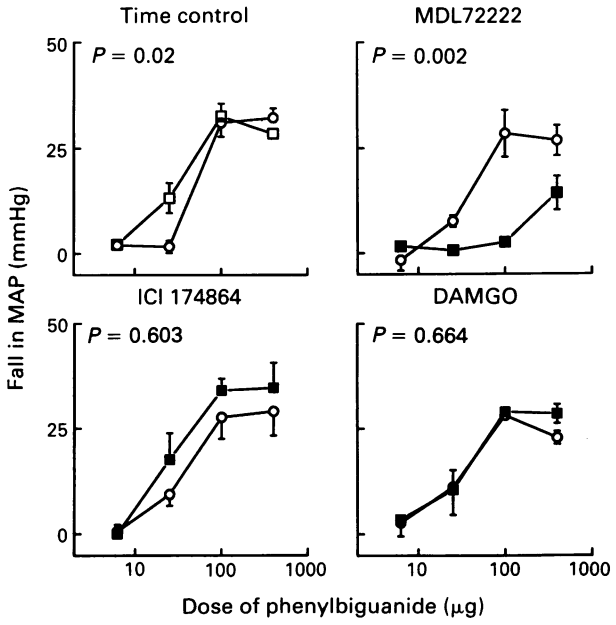

Figure 3 Dose-response relationships for left atrial phenylbiguanide according to the 4 studies, and treatments within studies. MAP, mean arterial pressure. Mean values for 3 rabbits. Vertical bars indicate 1 s.e.mean. Treatments are indicated by symbols: (○) no treatment (time control study 1) or vehicle (remaining studies). (□) No treatment (time control study 2); (■) i.v. MDL 72222 (1.0 mg kg^{-1}), 4th ventricular ICI 174864 (100 nmol), or 4th ventricular H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO, 100–300 pmol). *P* values attributable to dose × treatment interaction within ANOVA.

lated haemorrhage (Figure 4), but had no effects on the pattern of the responses of MAP to left atrial phenylbiguanide (Figure 3).

Right atrial phenylbiguanide protocol

Fourth ventricular ICI 174864 (100–300 nmol) and DAMGO (300 pmol) prevented the occurrence of Phase 2 of simulated haemorrhage (Figure 6), but had no effects on the pattern of the responses of MAP to right atrial phenylbiguanide (Figure 5).

Discussion

So far as we can ascertain, this experiment is the first to be described in which an attempt has been made to attribute a physiological phenomenon in a conscious animal to a signal transmitted by chemosensitive cardiac afferents. Our results are negative, in the sense that we have found no evidence that the cardiopulmanory afferents responsible for the signal that initiates the sympathoinhibitory phase of simulated haemorrhage in unanaesthetized rabbits correspond to those excited by atrial injection of phenylbiguanide or intrapericardial injection of nicotine.

We have confirmed our previous findings that, in conscious rabbits the sympathoinhibitory phase of simulated haemorrhage depends on a δ-opioid receptor mechanism within the central nervous system, and its expression can also be prevented by activating central μ-opioid receptors (Figures 2, 4 and 6) (Evans *et al.*, 1989b; Evans & Ludbrook, 1990). We have now shown that the reflex hypotension which follows excitation of

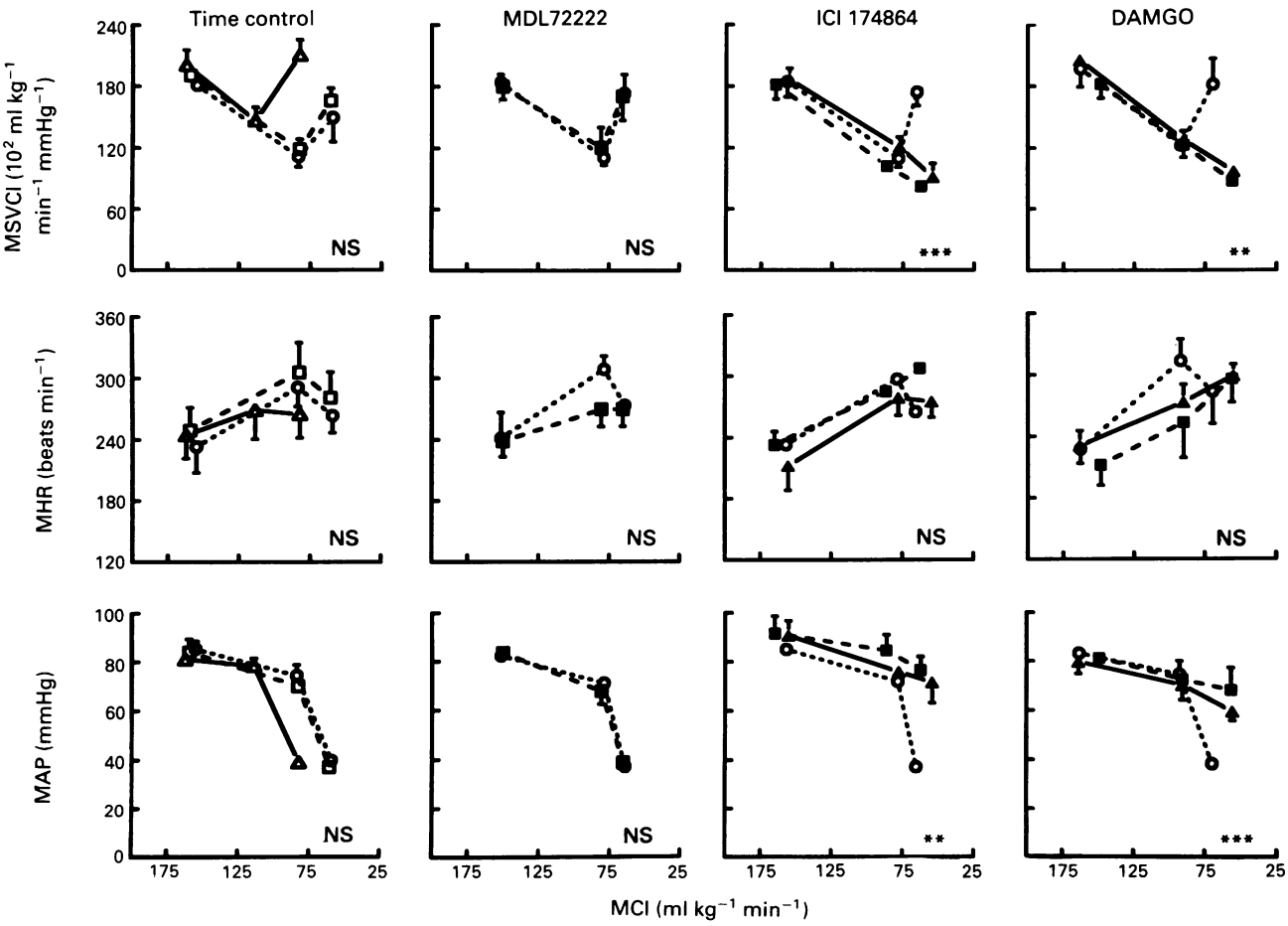

Figure 4 Haemodynamic effects of simulated haemorrhage in left atrial phenylbiguanide protocol according to studies within protocol and treatments within studies. Haemodynamic variables as in Figure 2. Symbols indicate mean coordinates for 3 rabbits. Vertical bars indicate 1 s.e.mean. Treatments are indicated by symbols: (○) no treatment (time control study No. 1) or vehicle treatment (remaining studies); (□) no treatment (time control study No. 2); (△) no treatment (time control study No 3); (■) i.v. MDL 72222 (1.0 mg kg^{-1}), 4th ventricular ICI 174864 (100 nmol), or 4th ventricular H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO, (100–300 pmol)); (▲) second treatment with ICI 174864 or DAMGO. Comparisons between levels at end of simulated haemorrhage were made by ANOVA. NS, $P \geqslant 0.05$; *$0.01 \leqslant P < 0.05$; **$0.001 \leqslant P < 0.01$; ***$P < 0.001$.

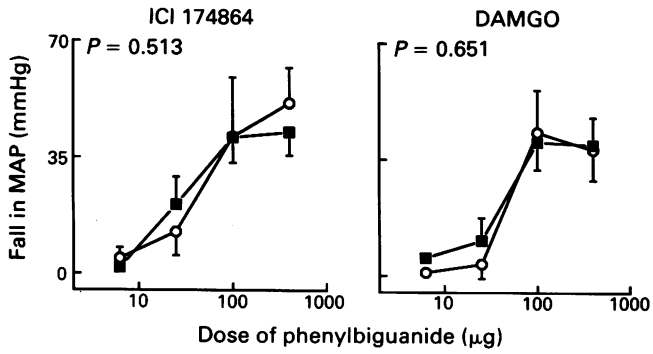

Figure 5 Dose-response relationships for right atrial phenylbiguanide according to the 2 studies, and treatments within studies. MAP, mean arterial pressure. Mean values for 3 rabbits. Vertical bars indicate 1 s.e.mean. Treatments are indicated by symbols: (○) saline treatment; (■) 4th ventricular ICI 174864 (100–300 nmol), or 4th ventricular H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO, 300 pmol). *P* values attributable to dose × treatment interaction within ANOVA.

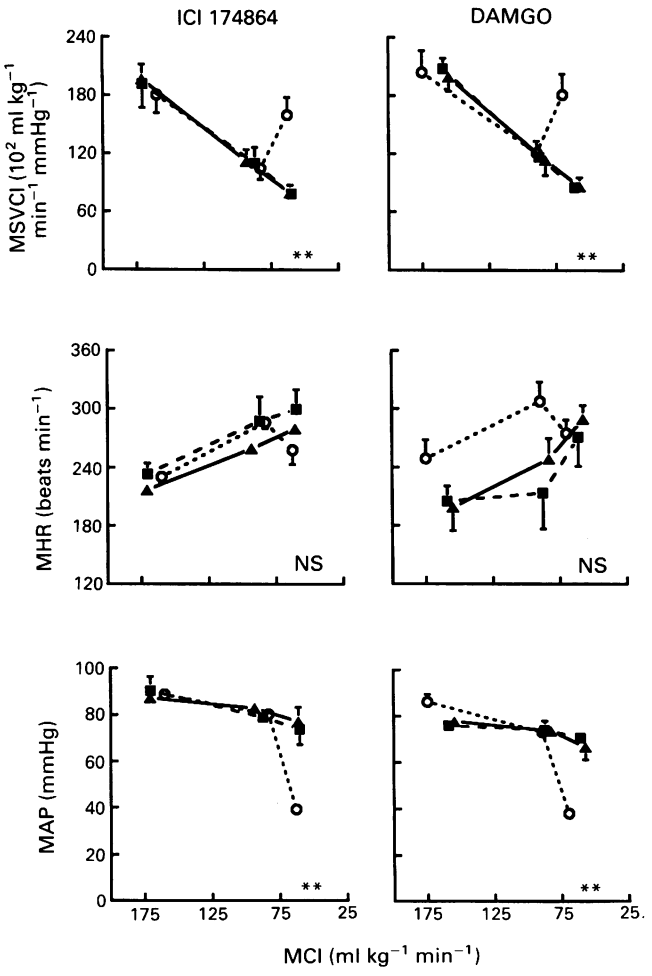

Figure 6 Haemodynamic effects of simulated haemorrhage in right atrial phenylbiguanide protocol according to the 2 studies, and treatments within studies Haemodynamic variables as in Figure 2. Symbols indicate mean coordinates for 3 rabbits. Vertical bars indicate 1 s.e.mean. Treatments are indicated by symbols: (○) vehicle treatment; (■) 4th ventricular ICI 174864 (100–300 nmol) or 4th ventricular H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO, 300 pmol); (▲) second treatment with ICI 174864 or DAMGO. Comparisons between levels at end of simulated haemorrhage were made by ANOVA. NS, $P \geqslant 0.05$; *$0.01 \leqslant P < 0.05$; **$0.001 \leqslant P < 0.01$.

the chemosensitive afferents does not depend on a δ-opioid receptor mechanism and is not modulated by central μ-opioid receptors (Figures 1, 3 and 5). In view of this finding, it is not surprising that the sympathoinhibitory phase of simulated haemorrhage was not prevented, delayed or attenuated by antagonizing the 5-HT_3 receptors responsible for the sensitivity of cardiac afferents to phenylbiguanide (Figure 4), or by antagonizing the neuronal-type nicotinic cholinoceptors responsible for the sensitivity of epicardial afferents to nicotine (Figure 2).

There are two possible criticisms of our conclusions. The first is a matter of the doses of the δ-antagonist and μ-agonist that we used as markers of the pathway followed by the input from cardiac afferents which is responsible for initiating the sympathoinhibitory phase of simulated haemorrhage. We are confident that the biophase levels of ICI 174864 and DAMGO were adequate at the time that the dose-response relationships for phenylbiguanide and nicotine were constructed, since we showed that the sympathoinhibitory phase was absent in a subsequent simulated haemorrhage (Figures 2, 4 and 6). Likewise, the effects of the 5-HT_3 antagonist MDL 72222 and the neuronal-type nicotinic cholinoceptor antagonist mecamylamine on simulated haemorrhage were tested before the chemoreflex dose-response relations were constructed. The dose-response relationships showed that the effects of phenylbiguanide and nicotine were still markedly antagonized (Figures 1 and 3).

The second possible criticism is as follows. Though we can be confident that the afferents responsible for the sympathoinhibitory phase of simulated haemorrhage do not correspond to the whole population of phenylbiguanide-sensitive cardiopulmonary afferents, or to the whole population of nicotine-sensitive epicardial afferents, we cannot exclude the possibility that they constitute a small sub-population of one or the other. If this were indeed so, effects of ICI 174864 or DAMGO on the pathway followed by the small sub-population might be concealed by lack of effects on the pathway followed by the larger sub-population.

We have already referred to the circumstantial evidence that phenylbiguanide-sensitive cardiopulmonary afferents might be responsible for the sympathoinhibitory phase of simulated haemorrhage (see Introduction). There is another piece of evidence that obliquely supports this hypothesis. Whereas cardiac afferents in rabbits (Burke & Dorward, 1988; Evans *et al.*, 1989a), and vagal afferents in rats (Skoog *et al.*, 1985), convey the signal responsible for the sympathoinhibitory phase of haemorrhage or simulated haemorrhage, in dogs this signal does not appear to travel in cardiac afferents (Shen *et al.*, 1990), and in primates the source of the signal is unknown (*see* Schadt & Ludbrook, 1990). In rabbits (Evans *et al.*, 1990a) and rats (Willette *et al.*, 1982) phenylbiguanide-sensitive cardiopulmonary afferents are prominent, whereas they appear to be absent in dogs (Dawes *et al.*, 1952) and man (Jain *et al.*, 1972). On the basis of these pieces of circumstantial evidence we had expected to find that in conscious rabbits the signal that initiates the sympathoinhibitory phase of simulated haemorrhage was conveyed by phenylbiguanide-sensitive cardiac afferents, but we have not been able to demonstrate that this is so. The nicotine-sensitive epicardial afferents are also mechanosensitive (Sleight & Widdicombe, 1965), and the sympathoinhibitory phase of haemorrhage has been attributed to paradoxical excitation of left ventricular mechanoreceptors (Thorén, 1979). Though the latter may be true, at least in rats and rabbits, we have been unable to demonstrate that these correspond to the nicotine-sensitive epicardial receptors.

We are grateful to Louise Gallagher and Giannina Legudi for their technical assistance with these experiments. This work was supported by the National Health and Medical Research Council of Australia and the National Heart Foundation of Australia.

References

BURKE, S.L. & DORWARD, P.K. (1988). Influence of endogenous opiates and cardiac afferents on renal nerve activity during haemorrhage in conscious rabbits. *J. Physiol.*, **402**, 9–27.

COTTON, R., GILES, M.G., MILLER, L., SHAW, J.S. & TIMMS, D. (1984). ICI 174864: a highly selective antagonist for the opioid δ-receptor. *Eur. J. Pharmacol.*, **97**, 331–332.

DAWES, G.S., MOTT, J.C. & WIDDICOMBE, J.G. (1952). Chemoreceptor reflexes in the dog and the action of phenyl diguanide. *Arch. Int. Pharmacodyn. Ther.*, **90**, 203–222.

EVANS, R.G. & LUDBROOK, J. (1990). Effects of μ-opioid receptor agonists on circulatory responses to simulated haemorrhage in conscious rabbits. *Br. J. Pharmacol.*, **100**, 421–426.

EVANS, R.G., LUDBROOK, J. & MICHALICEK, J. (1990a). Characteristics of cardiovascular reflexes originating from 5-HT_3 receptors in the heart and lungs of unanaesthetized rabbits. *Clin. Exp. Pharmacol. Physiol.*, **17**, 665–679.

EVANS, R.G., LUDBROOK, J. & POTOCNIK, S.J. (1989a). Intracisternal naloxone and cardiac nerve blockade prevent vasodilatation during simulated haemorrhage in awake rabbits. *J. Physiol.*, **409**, 1–14.

EVANS, R.G., LUDBROOK, J. & VAN LEEUWEN, A.F. (1989b). Role of central opiate receptor subtypes in the circulatory responses of awake rabbits to graded caval occlusions. *J. Physiol.*, **419**, 15–31.

EVANS, R.G., MICHALICEK, J. & LUDBROOK, J. (1990b). Excitation of 5-hydroxytryptamine$_3$ receptors and nicotinic ganglionic cholinoceptors on vagal cardiopulmonary afferents in rabbits. *Eur. J. Pharmacol.*, **183**, 1111.

FADEN, A.I. & HOLADAY, J.W. (1979). Opiate antagonists: a role in the treatment of hypovolemic shock. *Science*, **205**, 317–318.

FOZARD, J.R. (1984a). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 36–44.

FOZARD, J.R. (1984b). Neuronal 5-HT receptors in the periphery. *Neuropharmacology*, **23**, 1473–1486.

HANDA, B.K., LANE, A.C., LORD, J.A.H., MORGAN, B.A., RANCE, M.J. & SMITH, C.F.C. (1981). Analogues of β-LPH$_{61-64}$ possessing selective agonist activity at μ-opiate receptors. *Eur. J. Pharmacol.*, **70**, 531–540.

HIGUCHI, S., MORGAN, D.A. & MARK, A.L. (1988). Contrasting reflex effects of chemosensitive and mechanosensitive vagal afferents. *Hypertension*, **11**, 674–679.

JAIN, S.K., SUBRAMANIAN, S., JULKA, D.B. & GUZ, A. (1972). Search for evidence of lung chemoreflexes in man: study of respiratory and circulatory effects of phenyldiguanide and lobeline. *Clin. Sci.*, **42**, 163–177.

LUDBROOK, J. (1984). Comparison of the reflex effects of arterial baroreceptors and cardiac receptors on the heart rate of conscious rabbits. *Clin. Exp. Pharmacol. Physiol.*, **11**, 245–260.

LUDBROOK, J. & GRAHAM, W.F. (1984). The role of cardiac receptor and arterial baroreceptor reflexes in the control of the circulation during acute change of blood volume in the conscious rabbit. *Circ. Res.*, **54**, 424–435.

LUDBROOK, J., POTOCNIK, S.J. & WOODS, R.L. (1988). Simulation of acute haemorrhage in unanaesthetized rabbits. *Clin. Exp. Pharmacol. Physiol.*, **15**, 575–584.

LUDBROOK, J. & RUTTER, P.C. (1988). Effect of naloxone on haemodynamic responses to acute blood loss in unanaesthetized rabbits. *J. Physiol.*, **400**, 1–14.

MORITA, H., NISHIDA, Y., MOTOCHIGAWA, H., UEREMA, N., HOSOMI, H. & VATNER, S.F. (1988). Opiate receptor-mediated decrease in renal nerve activity during hypotensive hemorrhage in conscious rabbits. *Circ. Res.*, **63**, 165–172.

SCHADT, J.C. & LUDBROOK, J. (1990). Hemodynamic and neurohumoral responses to acute hyporolemia in conscious mammals. *Am. J. Physiol.* (in press).

SCHADT, J.C., McKOWN, M.D., McKOWN, D.P. & FRANKLIN, D. (1984). Hemodynamic effects of hemorrhage and subsequent naloxone treatment in conscious rabits. *Am. J. Physiol.*, **247**, R497–R508.

SHEN, Y-T., KNIGHT, D.R., THOMAS, J.X. & VATNER, S.F. (1990). Relative roles of cardiac receptors and arterial baroreceptors during hemorrhage in conscious dogs. *Cir. Res.*, **66**, 397–405.

SHEN, Y-T., KNIGHT, D.R., THOMAS, J.X. & VATNER, S.F. (1990). Relative roles of cardiac receptors and arterial baroreceptors during haemorrhage in conscious dogs. *Cir. Res.*, **66**, 397–405.

SKOOG, P., MÅNSSON, J. & THORÉN, P. (1985). Changes in renal sympathetic outflow during hypotensive haemorrhage in rats. *Acta Physiol. Scand.*, **125**, 655–660.

SLEIGHT, P. & WIDDICOMBE, J.G. (1965). Action potentials in fibres from receptors in the epicardium and myocardium of the dog's left ventricle. *J. Physiol.*, **181**, 235–258.

THORÉN, P. (1979). Role of cardiac vagal C-fibres in cardiovascular control. *Rev. Physiol. Biochem. Pharmacol.*, **86**, 1–94.

VAN ROSSUM, J.M. (1962). Classification and molecular pharmacology of ganglionic blocking agents. II. Mode of action of competitive and non-competitive ganglionic blocking agents. *Int. J. Neuropharmacol.*, **1**, 401–421.

VICTOR, R.G., THORÉN, P. & MARK, A.L. (1989). Differential control of adrenal and renal sympathetic nerve activity during hemorrhagic hypotension in rats. *Circ. Res.*, **64**, 686–694.

WILLETTE, R.N., GATTI, P., GERTNER, S.B. & SAPRU, H.N. (1982). Pulmonary vagal afferent stimulants in the conscious rat: opioids and phenyldiguanide. *Pharmacol. Biochem. Behav.*, **17**, 19–23.

WRIGHT, C.E. & ANGUS, J.A. (1989). 5-Carboxyamidotryptamine elicits 5-HT_2 and 5-HT_3 receptor-mediated responses in the conscious rabbit: evidence for 5-HT release from platelets. *J. Cardiovasc. Pharmacol.*, **13**, 557–564.

(Received June 21, 1990
Revised September 3, 1990
Accepted October 4, 1990)