The effects of hypertonic saline solution on coronary blood flow in anaesthetized pigs

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1. The effects of intracoronary bolus infusion of hypertonic saline solution on left circumflex coronary blood flow were examined in sixteen anaesthetized and artificially ventilated pigs whilst preventing changes in heart rate and arterial blood pressure.

2. In fourteen pigs, bolus infusion of 7·5% hypertonic saline solution (2 ml within 30 s) caused a steady-state increase in coronary blood flow without significantly affecting right atrial or left ventricular pressure and its rate of rise (dP/dt_{max}). Infusing normal saline solution (0·9%) at the same rate and volume in seven pigs did not have this effect.

3. In five pigs, the magnitude and the duration of the response of increase in coronary blood flow were increased in a graded manner by graded increases in the concentration of the hypertonic saline solution between 2·5, 5 and 7·5%.

4. In nine pigs, the response of increase in coronary blood flow to the bolus infusion of hypertonic saline solution was not affected by the blocking agents atropine, propranolol and phentolamine, but it was completely abolished in the same nine pigs by the subsequent intracoronary administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME) which blocks the synthesis of endothelium-derived relaxing factor (EDRF) and in seven pigs by solely giving L-NAME.

5. These results showed that the intracoronary bolus infusion of hypertonic saline solution in anaesthetized pigs caused a coronary vasodilatation which involved mechanisms dependent on the release of EDRF.

Intravenous administration of small volumes of 7·5% hypertonic saline solution (2400 mosmol l^{-1}) has been used as a rapid treatment for hypovolaemic shock in animals and in man (De Felippe, Timoner, Velasco, Lopes & Rocha e Silva, 1980; Velasco, Pontieri, Rocha e Silva & Lopes, 1980; Nakayama, Sibley, Gunther, Holcroft & Kramer, 1984; Smith, Kramer, Perron, Nakayama, Gunther & Holcroft, 1985; Rocha e Silva, Velasco, Nogueira Da Silva, Oliveira, Negraes & Oliveira, 1987; Pascual, Watson, Runyon, Wade & Kramer, 1992). This has been reported to involve movement of fluid from the interstitial spaces into the intravascular compartment with an increase in venous return, improvement of cardiac performance and reduction of ventricular after-load (Gazitua, Scott, Swindall & Haddy, 1971; Schertel, Valentine, Rademakers & Muir, 1990; Kien, Reitan, White, Wu & Eisele, 1991; Pascual *et al.* 1992).

The improvement in blood flow caused by hypertonic saline solutions in regional vascular beds has been studied in experimentally induced haemorrhagic shock in animals (Rocha e Silva, Negraes, Soares, Pontieri & Loppnow, 1986; Maningas, 1987; Kreimeier, Bruckner, Niemczyk & Messmer, 1990). In these studies, systemic infusion of hypertonic saline solution was shown to elicit a remarkable and sustained increase in myocardial blood flow. However, this was accompanied by increases in aortic blood pressure and left ventricular inotropic state, which could have secondarily increased coronary blood flow and therefore prevented determination of the direct effects of hypertonic saline solutions on this flow. Indeed, the available reports on the direct effects of hypertonic saline solutions on coronary blood flow suggest that they cause only a small and transient change in flow (Crystal, Gurevicius, Kim, Eckel, Ismail & Salem, 1994).

The present work was planned to study, in anaesthetized pigs, the direct effect of the intracoronary bolus infusion of a small volume of hypertonic saline solution and the mechanism of its effect. This was achieved by comparing the changes in left circumflex coronary blood flow induced by intracoronary administration of 2 ml hypertonic saline solution with the changes in blood flow induced by normal saline whilst keeping heart rate and arterial blood pressure

constant to prevent the secondary interference of changes in these variables. The effects of graded changes in the concentration of the hypertonic saline solution on coronary blood flow were investigated and tests were made of the role of nitric oxide in the induced changes in coronary blood flow.

METHODS

The experiments were carried out in sixteen pigs weighing 68–74 kg. The animals, which were fasted overnight, were anaesthetized with ketamine (20 mg kg^{-1} I.M., Parke-Davis, Milan, Italy) and, about 15 min later, sodium pentobarbitone (15 mg kg^{-1} I.V., Siegfried Zofingen, Switzerland). The anaesthetized pigs were artificially ventilated with oxygen-enriched air (approximately 40%) with a respiratory pump (Harvard 613; Harvard Apparatus, South Natick, MA, USA). During surgical preparation and the experiments, anaesthesia was maintained by continuous infusion of sodium pentobarbitone (7 mg kg^{-1} h^{-1} I.V.) and assessed as previously reported (Linden & May, 1983), judging from responses of the animals to somatic stimuli.

Pressures in the ascending aorta and in the right atrium were recorded using two catheters connected to pressure transducers (Statham P23 XL; Gould, Valley View, OH, USA) inserted into the right femoral artery and the right external jugular vein, respectively. The chest was opened in the left fourth intercostal space and an electromagnetic flowmeter (Model BL 613; Biotronex Laboratory Inc., Chester, MD, USA) probe was positioned around the proximal part of the left circumflex coronary artery. A plastic snare was placed around the artery distal to the probe for the assessment of zero blood flow. A catheter was inserted into a small artery originating in the marginal or oblique branch of the left circumflex coronary artery and used for intracoronary infusion of hypertonic saline solution. Left ventricular pressure was measured by means of a catheter inserted through the left atrium. To pace the heart, electrodes were sewn on the left atrial appendage and connected to a stimulator (Model S8800; Grass Instruments, Quincy, MA, USA) which delivered pulses of 3–5 V with 2 ms duration at the required frequency.

A plastic snare was placed around the thoracic aorta close to the diaphragm. Changes in blood pressure in the thoracic aorta were prevented in the control periods and during the experiments by performing a moderate aortic constriction. Arterial blood samples were used to measure pH, P_{O_2} and P_{CO_2}. The acid–base status of the animals was kept within normal limits as previously described (Linden & Mary, 1983). Blood coagulation was avoided by injection of heparin (Parke-Davis, initial dose 500 i.u. kg^{-1} I.V., subsequent doses of 50 i.u. kg^{-1} I.V. every 30 min). During the experiments the rectal temperature of the pigs was monitored and kept constant at 38–40 °C using an electric pad.

Mean aortic and right atrial pressures, left ventricular pressure, mean and phasic coronary blood flow were monitored and recorded together with heart rate and the maximum rate of change of left ventricular pressure (dP/dt_{max}) using an electrostatic strip chart recorder (Gould ES 2000). The heart rate was obtained from the electrocardiogram. For the purpose of calculating coronary vascular resistance (CVR), the pressure gradient was obtained as the difference between mean aortic pressure and mean left ventricular pressure during diastole. The latter was defined as starting when blood pressure reached its minimum value after systole. CVR was calculated as the ratio between this pressure gradient and mean diastolic coronary blood flow.

At the end of the experiments, each animal was killed by intravenous injection of 90 mg kg^{-1} sodium pentobarbitone.

Experimental protocol

The experiments were performed after a steady state had been attained for at least 30 min with respect to heart rate, mean aortic and right atrial pressures, mean coronary blood flow, left ventricular pressures and dP/dt_{max}.

In all sixteen pigs, to avoid any possible changes in heart rate and arterial blood pressure during the experiments, the heart was paced to a frequency higher, by 24·1 ± 14·6 beats min^{-1} (range, 5–55 beats min^{-1}), than that observed in the steady state and mean aortic blood pressure was increased by 9·3 ± 4·9 mmHg (4–18) using the aortic snare. After at least 10 min of steady-state conditions, the experiments were carried out by infusing into the coronary artery 2 ml of a 7·5% saline solution (at 38 °C) in a period of 30 s using an infusion pump (Model 22, Harvard Apparatus) working at constant rate of 4 ml min^{-1}. In seven pigs, to determine the possible mechanical effects of the bolus infusion, 2 ml of isotonic saline solution (0·9% sodium chloride) were administered at the same rate. The effect of graded changes in the concentration of the solution on coronary blood flow was examined in five animals by administering in a random order an equal volume of 2·5, 5 and 7·5% hypertonic saline solutions.

The pattern of changes in measured variables was examined in all the animals during the bolus infusion of solutions and after stopping the infusions. During the bolus infusions a peak increase in coronary blood flow was observed in each animal. The peak increase in coronary blood flow during the bolus infusion of hypertonic saline solutions was compared to that obtained during the bolus infusion of normal saline. After stopping the infusion, we identified the 'test' period in which we made our assessments as beginning when coronary blood flow reached a steady level and ending when it declined; the latter depended on the concentration of the saline solution used (see Results). Two control periods, each of 3 min were taken, immediately before starting the infusion and 30 min after its end. The response of coronary blood flow to the bolus infusions was calculated as the difference between its mean value during the test period and the average of its values during the two control periods.

To investigate the mechanisms of the response of coronary blood flow, in nine of fourteen animals the experiment was repeated when a steady state was attained after the intravenous administration of the blocking agents atropine sulphate (0·5 mg kg^{-1}; Sigma), propranolol (0·5 mg kg^{-1}; Sigma) and phentolamine (1 mg kg^{-1}; Ciba-Geigy) and then was repeated again after the intracoronary administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 mg; Sigma). In the remaining five pigs the experiment was repeated after L-NAME only was given. In a further two pigs, bolus infusion of hypertonic saline was given only after the administration of L-NAME. The dose of L-NAME used was shown to significantly reduce the vasodilator effect of the intracoronary administration of acetylcholine at a dose of 1 μg (Gattullo, Pagliaro & Dalla Valle, 1994). In five of the sixteen pigs, this dose increased coronary blood flow at constant heart rate and arterial blood pressure from 39·8 ± 6 ml min^{-1} (31·9–46) to 86·4 ± 11·6 ml min^{-1} (72–101·3). When the same dose was given after L-NAME, coronary blood flow changed from 39·7 ± 5·5 ml min^{-1} (32–46·5) to 62·8 ± 11·6 ml min^{-1} (52–81·4), with a reduction in the response of 59·7% ± 9·9 (45·2–68·9, $P < 0·0005$). This reduction in the acetylcholine-induced increase in coronary blood flow was considered a reliable marker of the inhibition of the

Table 1. Control values of haemodynamic variables for the experiments of intracoronary bolus infusion of hypertonic saline solution

Experiment	Heart rate (beats min^{-1})	ABP (mmHg)	LVEDP (mmHg)	RAP (mmHg)	dP/dt (mmHg s^{-1})
Control	125 ± 15·4 (100–150)	102 ± 12·2 (85–130)	6·2 ± 1·5 (3–9·2)	3·2 ± 1·0 (1–5·2)	2295 ± 328 (1750–2964)
After blockade	123 ± 17·9 (100–150)	98 ± 14·6 (84–130)	7·2 ± 2·2 (4–10·3)	3·7 ± 1·2 (1·8–5·7)	1941 ± 361 (1443–2306)
After L-NAME	125 ± 14·5 (100–150)	110 ± 13·3 (92–146)	7 ± 1·8 (4–10·2)	3·6 ± 1·2 (2–6)	2121 ± 269 (1510–2490)

Data are means ± S.D. (range) for $n = 14$, 9 (after blockade with atropine, propranolol and phentolamine) and 16 (after L-NAME). ABP, mean aortic blood pressure; LVEDP, left ventricular end-diastolic pressure; RAP, mean right atrial pressure; dP/dt, left ventricular dP/dt_{max}.

release of endothelium-derived relaxing factor (EDRF; Parent, Paré & Lavallée, 1992).

The administration of the blocking agents was performed at least 30 min after coronary blood flow had returned to the same control level observed in each pig before the previous bolus infusion of hypertonic saline solution. In the experiments performed after giving these drugs, the heart was paced to the same frequency as in the previous experiments. In some pigs, the bolus infusion of hypertonic saline solution after giving L-NAME was repeated following the intracoronary administration of 0·1 mg of nitroprusside, which was given at this dose to reduce the increase in coronary tone induced by L-NAME.

Student's paired t test was used to examine changes caused by hypertonic saline solution in measured haemodynamic variables. The relationship between the concentration of hypertonic saline solution and changes in mean coronary blood flow was examined using least-square procedures for linear correlation analysis. Analysis of variance was used to examine the effect of successive procedures on coronary blood flow. Group data are presented as means ± S.D. (range).

RESULTS

In the sixteen pigs, recordings commenced approximately 5 h after the induction of anaesthesia. The mean pH, P_{O_2} and P_{CO_2} of arterial blood were 7·41 (7·38–7·45), 118 mmHg (106–163) and 38 mmHg (36–42), respectively and the haematocrit was 35% (32–39).

Effect of infusion of hypertonic saline solution

The effect of the intracoronary bolus infusion of 7·5% saline solution was examined in fourteen animals, seven of which also received normal saline. In five of these seven animals, saline solutions at 2·5 and 5% were also given. The spontaneous heart rate and mean aortic blood pressure were 98·6 ± 12·6 beats min^{-1} (76–125) and 91·3 ± 11·8 mmHg (78–126) and left ventricular dP/dt_{max} and mean coronary blood flow were 2031 ± 212 mmHg s^{-1} (1500–2362) and 39·7 ± 7·6 ml min^{-1} (26·5–53).

Control values of haemodynamic variables (in the presence of atrial pacing and aortic constriction) are shown in Table 1. The response of mean coronary blood flow during the test period is given in Table 2. In the fourteen pigs examined, during the intracoronary bolus infusion of 7·5% saline solution, an increase in mean coronary blood flow of 29·7 ± 7·7 ml min^{-1} (17·9–39) was obtained. A similar increase in coronary blood flow of 27·6 ± 6·9 ml min^{-1} (20–37·2) was caused in seven pigs by the bolus infusion of normal saline. The difference between this response and the response observed in the same seven pigs during the bolus infusion of hypertonic saline solution was insignificant ($P > 0·35$). When the infusion of normal saline was stopped, coronary blood flow returned to the initial control values in a few seconds, while when the infusion of 7·5% saline solution was stopped, mean coronary blood flow, after an initial decrease, maintained a steady-state increase (Table 2) which amounted to 28 ± 12% (11·8–55·2) of the control values. This steady-state increase in mean coronary blood flow was sustained for a period of 13·9 ± 3·6 min (11–23) and then coronary blood flow began to decrease toward the control values. Changes in the other haemodynamic variables during the test period of the experiments were insignificant (at least $P > 0·10$). An example of this response is shown in Fig. 1. In this experiment, the bolus infusion of hypertonic saline solution caused a steady-state increase in mean coronary blood flow of 16·8 ml min^{-1} from a control value of 40·5 ml min^{-1}. Individual responses of coronary blood flow in the fourteen animals are shown in Fig. 2.

In the five pigs in which the effect of graded concentrations of saline solution was examined, the peak increase in mean coronary blood flow during the bolus infusion of 2·5, 5 and 7·5% saline solutions was not significantly different from the peak increase caused by bolus infusion of normal saline (at least $P > 0·25$). After stopping the infusions of hypertonic saline solutions, a steady-state increase in mean coronary blood flow was attained in each of the five pigs, being greater and longer lasting with the higher values of concentration of the saline solution. The increases in mean coronary blood flow for the hypertonic saline solutions of

Table 2. Changes in mean coronary blood flow caused by the intracoronary bolus infusion of hypertonic saline solution

Experiment	Average control	Test	Change	P
Control	44·7 ± 7·4 (30·8–55·1)	56·7 ± 7·3 (43·0–68·9)	12·0 ± 4·1 (6·5–20·8)	< 0·0005
After blockade	42·3 ± 7·1 (27·2–50·2)	54·5 ± 5·7 (44·2–62·4)	12·2 ± 3·5 (8–17)	< 0·0005
After L-NAME	43·7 ± 7·6 (27·5–56·7)	43·8 ± 7·5 (28·2–56·7)	0·1 ± 0·6 (−1·1 to 1·2)	> 0·25

Data, expressed in millilitres per minute, are means ± S.D. (range) for $n = 14$, 9 (after blockade with atropine, propranolol and phentolamine) and 16 (after L-NAME).

2·5, 5 and 7·5% were 3·2 ± 0·7 (2·6–4·5, $P < 0·0005$), 6 ± 1·6 (4·3–8·4, $P < 0·0025$) and 10·4 ± 2·5 ml min^{-1} (7·7–14, $P < 0·0005$), respectively, and were sustained for 5·4 ± 1·3 (4–7), 10 ± 2·5 (8–16) and 14·2 ± 3·5 min (11–20), respectively. The increase in mean coronary blood flow caused by 7·5% saline solution was significantly higher ($P < 0·025$) and lasted significantly longer ($P < 0·0025$) than the increase caused by 5% saline solution. The latter was significantly higher ($P < 0·0025$) and lasted significantly longer ($P < 0·0025$) than that elicited by 2·5% saline solution. Considering all the animals, there was a significant linear relation ($r = 0·88$, $P < 0·0005$) between the increase in mean coronary blood flow and the concentration of the hypertonic saline solution. An example of the coronary changes caused by normal saline and by the three hypertonic saline solutions in one animal is shown in Fig. 3. In this pig, the steady-state increase in mean coronary blood flow for 2·5, 5 and 7·5% saline solutions amounted to 2·6, 4·3 and 11 ml min^{-1}, respectively. The duration of these increases was 7, 10 and 14 min, respectively. Individual responses of coronary blood flow to the graded increase in hypertonic saline solution concentration in the five pigs are illustrated in Fig. 4.

Mechanisms of the response of coronary blood flow

The mechanisms responsible for the increase in coronary blood flow due to intracoronary bolus infusion of hypertonic saline solution were examined by performing intracoronary administration of 7·5% saline solution after blockade of coronary cholinergic and adrenergic receptors

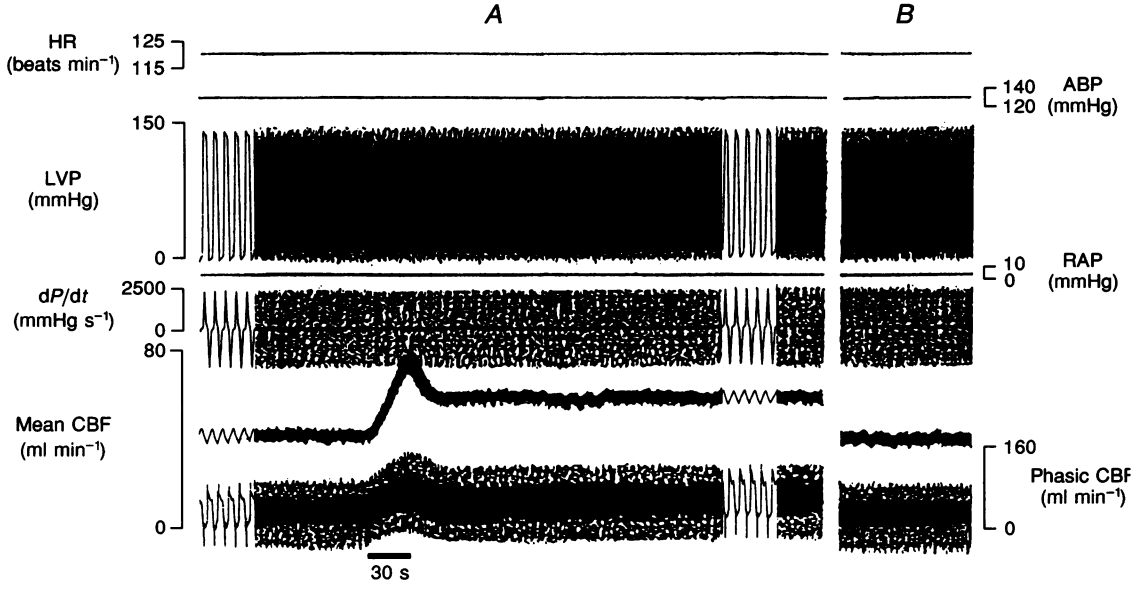

Figure 1

Example of experimental recordings taken during an experiment of intracoronary bolus infusion of hypertonic saline solution (*A*) and 30 min after its end (*B*) in an anaesthetized pig. The bar at the bottom indicates the 30 s period of infusion. From the top are shown heart rate (HR), mean aortic blood pressure (ABP), left ventricular pressure (LVP), mean right atrial pressure (RAP), left ventricular dP/dt_{max} (dP/dt), mean and phasic coronary blood flow (CBF).

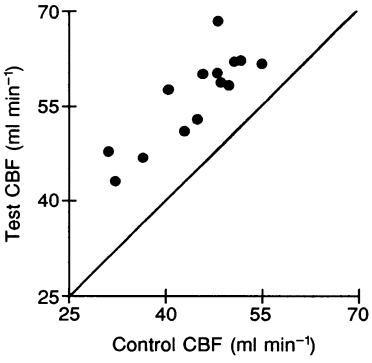

Figure 2. The steady-state response of mean coronary blood flow (CBF) to the intracoronary bolus infusion of hypertonic saline solution in fourteen pigs

The values obtained during the test period after the infusion are plotted on the ordinate against the control values on the abscissa. The continuous line is the line of equality.

with atropine, propranolol and phentolamine and after the intracoronary injection of L-NAME.

Experiments with atropine, propranolol and phentolamine

The administration of these blocking agents in the nine pigs caused decreases in heart rate and mean aortic blood pressure of 13·2 ± 8·7 beats min^{-1} (−28 to 3, $P < 0·0025$) and of 10·8 ± 14·7 mmHg (−32 to 4, $P < 0·05$), respectively. These changes were accompanied by decreases in left ventricular dP/dt_{max} and mean coronary blood flow which respectively amounted to 345 ± 255 mmHg s^{-1} (−746 to 63, $P < 0·0025$) and to 6·1 ± 7·5 ml min^{-1} (−24·3 to 0·9, $P < 0·025$) and by an increase in CVR of 0·15 ± 0·46 mmHg ml^{-1} min^{-1} (−0·6 to 1·05, $P > 0·15$).

Control values of haemodynamic variables are given in Table 1. The response of mean coronary blood flow to the bolus infusion of hypertonic saline solution is given in Table 2. The administration of the blocking agents did not affect the response of coronary blood flow to the bolus infusion. The difference in the increase in mean coronary blood flow caused by the bolus infusion of hypertonic saline solution before and after the administration of the blocking agents amounted to 0·6 ± 4·3 ml min^{-1} (−7·8 to 8·4, $P > 0·30$) and corresponded to a mean change of 1·6% (see

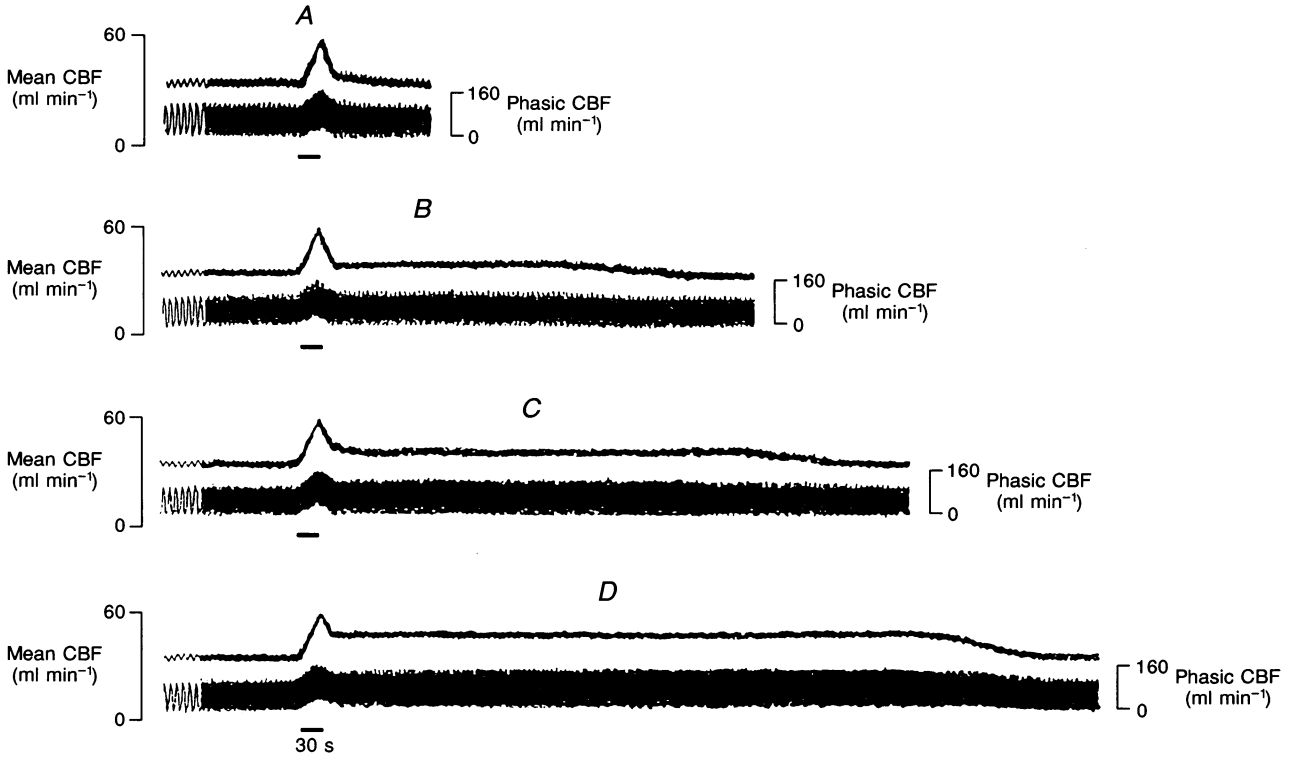

Figure 3

Examples of experimental recordings during intracoronary bolus infusion of normal (*A*), 2·5 (*B*), 5 (*C*) and 7·5% (*D*) saline solutions on coronary blood flow (CBF) in an anaesthetized pig. The bars indicate the periods of infusion.

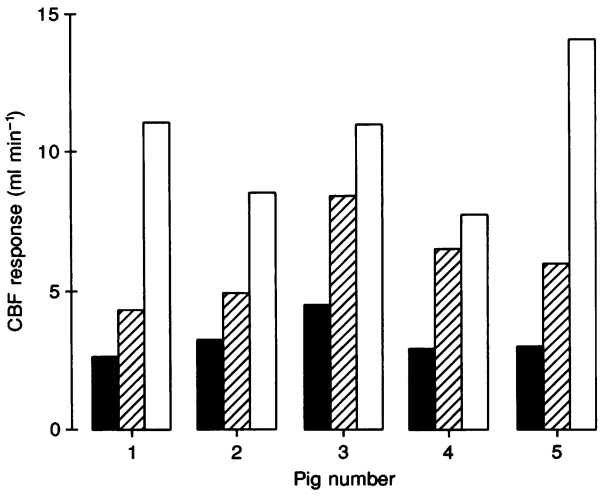

Figure 4

Responses of mean coronary blood flow (CBF) to the intracoronary bolus infusion of 2·5 (■), 5 (▨) and 7·5% (□) saline solutions in 5 pigs (numbered 1 to 5).

Fig. 5). The difference in the duration of the response of mean coronary blood flow before and after the administration of the drugs was also insignificant ($P > 0{\cdot}35$). Changes in the other haemodynamic variables during the experiments were insignificant (at least $P > 0{\cdot}15$).

Experiments with L-NAME

In the sixteen pigs, the intracoronary injection of L-NAME caused an increase in mean aortic blood pressure of $14{\cdot}3 \pm 6$ mmHg (9–30, $P < 0{\cdot}0005$). This increase was accompanied by a decrease in heart rate of $6{\cdot}6 \pm 2{\cdot}8$ beats min^{-1} (3–12, $P < 0{\cdot}0005$), an increase in left ventricular dP/dt_{max} of 59 ± 50 mmHg s^{-1} (−20 to 180, $P < 0{\cdot}0005$), a change in mean coronary blood flow of $-0{\cdot}5 \pm 3{\cdot}1$ ml min^{-1} (−5·1 to 5·7, $P > 0{\cdot}25$) and an increase in CVR of $0{\cdot}43 \pm 0{\cdot}24$ mmHg ml^{-1} min^{-1} (−0·07 to 0·89, $P < 0{\cdot}0005$).

Control values of haemodynamic variables are given in Table 1. The response of mean coronary blood flow to the bolus infusion of hypertonic saline solution is given in Table 2. In the sixteen pigs, the administration of L-NAME completely abolished the increase in mean coronary blood flow elicited by hypertonic saline solution. Changes in the other haemodynamic variables during the experiments were insignificant (at least $P > 0{\cdot}10$). Considering the group of nine of the sixteen pigs in which

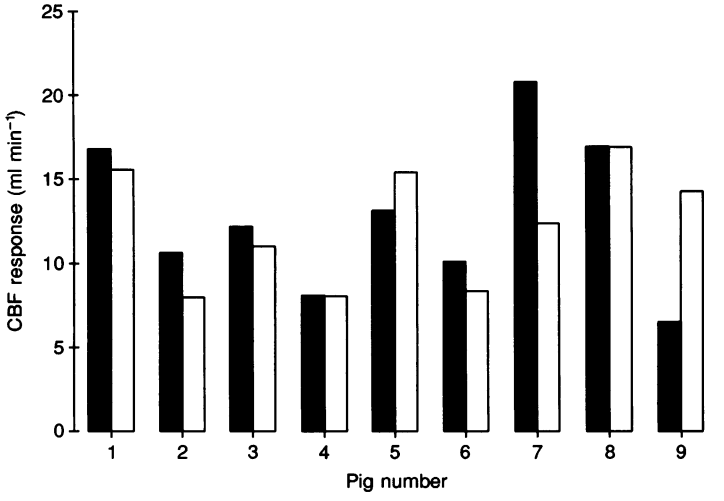

Figure 5

Responses of mean coronary blood flow (CBF) to the intracoronary bolus infusion of hypertonic saline solution before (■) and after (□) the administration of atropine, propranolol and phentolamine in 9 pigs (numbered 1–9).

the administration of L-NAME was performed after blocking coronary cholinergic and adrenergic receptors and the group of five pigs which received only L-NAME, changes in mean coronary blood flow to the bolus infusion of hypertonic saline solution after L-NAME were insignificant ($P > 0{\cdot}47$ and $P > 0{\cdot}10$, respectively). Also, analysis of variance for repeated measurements performed for the group of nine animals showed that this effect of L-NAME was significant ($F = 9{\cdot}0$, $P < 0{\cdot}0005$). In addition, no changes in mean coronary blood flow were caused by hypertonic saline solution in the two pigs in which the bolus infusion of the saline solution was performed only after the administration of L-NAME.

To find out whether the increase in the baseline value of coronary vascular resistance caused by L-NAME could itself affect the response of coronary blood flow to the hypertonic saline solution, in five pigs, the bolus infusion was repeated after the intracoronary administration of nitroprusside. The administration of nitroprusside caused a decrease in mean aortic blood pressure of $7{\cdot}4 \pm 2{\cdot}9$ mmHg (5–11, $P < 0{\cdot}0025$), an increase in heart rate of $4{\cdot}9 \pm 2{\cdot}4$ beats min^{-1} (3–9, $P < 0{\cdot}01$), a decrease in left ventricular dP/dt_{max} of 36 ± 40 mmHg s^{-1} (0–100, $P > 0{\cdot}05$) and an increase in mean coronary blood flow of $5{\cdot}2 \pm 1{\cdot}6$ ml min^{-1} (3–7, $P < 0{\cdot}0025$). The experiments performed after giving nitroprusside were carried out whilst pacing the heart to the same frequency and keeping arterial blood pressure at the same level as was recorded during the experiments after giving L-NAME. Under these experimental conditions, coronary vascular resistance, which was increased by L-NAME from a control value of $2{\cdot}44 \pm 0{\cdot}58$ (2·01–3·44) to a value of $2{\cdot}76 \pm 0{\cdot}58$ (2·34–3·75), was $2{\cdot}17 \pm 0{\cdot}27$ mmHg ml^{-1} min^{-1} (1·84–2·6) after nitroprusside. This reduction in coronary tone did not alter the effect of the bolus infusion of hypertonic saline solution. In the five pigs, mean coronary blood flow did not change significantly: after the infusion $0{\cdot}2 \pm 0{\cdot}6$ ml min^{-1} (−0·5 to 0·9, $P > 0{\cdot}20$), from a control value of $49{\cdot}8 \pm 5{\cdot}6$ ml min^{-1} (42·3–56·4). In these animals, hypertonic saline induced an increase in mean coronary blood flow of $10{\cdot}4 \pm 2{\cdot}5$ ml min^{-1} (7·7–14) before L-NAME ($P < 0{\cdot}0005$), but no significant change after L-NAME, but before nitroprusside: $0{\cdot}4 \pm 0{\cdot}7$ ml min^{-1} (−0·5 to 1·2, $P > 0{\cdot}10$).

DISCUSSION

The main findings of the present investigation were that the intracoronary infusion of a small volume of hypertonic saline solution caused a steady-state increase in coronary blood flow which was sustained for periods of time ranging from 11 to 23 min after stopping the infusion. Further, this steady-state increase and its duration were augmented by increasing the concentration of the hypertonic saline solution. However, this increase was not affected by blockade of coronary cholinergic and adrenergic receptors, but it was abolished by the intracoronary administration of L-NAME, indicating that the mechanism for the increase in coronary blood flow involved an increase in the release of EDRF.

That the increase in coronary blood flow was a primary response to intracoronary bolus infusion of the hypertonic saline solution was confirmed in several ways. Firstly, the effect of the infusion was studied whilst preventing changes in heart rate and arterial blood pressure, which could secondarily interfere with the response of coronary blood flow. Secondly, the bolus infusion of hypertonic saline solution did not cause any significant change in left ventricular pressure and dP/dt_{max}, indicating the absence of changes in left ventricular contractility which could have influenced coronary blood flow. Thirdly, the absence of changes in left ventricular filling excluded any significant interference by reflexes related to cardiovascular receptors. Finally, it was possible to grade the magnitude and the duration of the response of increase in coronary blood flow by increasing the concentration of the hypertonic saline solution. The present finding of a coronary vasodilatation following the administration of hypertonic saline solution contrasts with previous reports that anaesthetized dogs showed only a small and transient increase in coronary blood flow (Crystal *et al.* 1994). However, in that investigation the coronary circulation was perfused with blood and coronary blood flow was represented by the rate of flow of the perfused blood.

In the present investigation the coronary vasodilatation caused by the bolus infusion of hypertonic saline solution took several minutes to disappear in each pig. The same vasodilatation was elicited by hypertonic saline solution after blockade of coronary cholinergic receptors with atropine and of adrenergic receptors with propranolol and phentolamine. The dose of atropine used has been shown to block the vagally mediated component of the reflex changes in coronary blood flow caused by urinary bladder distension (Cevese, Drinkhill, Mary, Patel, Schena & Vacca, 1991). The dose of propranolol has been shown in anaesthetized dogs to prevent changes in left ventricular inotropic state (Harry, Kappagoda, Linden & Snow, 1973) and to abolish the reflex increases in heart rate and left ventricular dP/dt_{max} caused by descending colon distension (Cevese, Mary, Poltronieri, Schena & Vacca, 1992) and has previously been used to block β coronary receptors (Broten, Miyashiro, Moncada & Feigl, 1992). The same dose has been shown in anaesthetized pigs to prevent the reflex increases in heart rate caused by distension of the stomach (Vacca, Battaglia, Grossini, Mary, Papillo & Pelosi, 1994) and distension of the gallbladder (Vacca, Battaglia, Grossini & Papillo, 1994). A dose of 1 mg kg^{-1} phentolamine was shown to abolish the reflex increase in aortic blood pressure to descending colon distension in anaesthetized dogs (Cevese *et al.* 1992); in anaesthetized pigs this dose of phentolamine prevented the reflex renal

vasoconstriction caused by gastric distension (Vacca *et al.* 1994) and the reflex increase in aortic blood pressure caused by gallbladder distension (Vacca *et al.* 1994); in another report in anaesthetized dogs (Broten *et al.* 1992), a dose of 300 $\mu g\ kg^{-1}$ phentolamine was used to block α coronary receptors. The present findings therefore excluded the involvement of coronary cholinergic and adrenergic receptors, or of any possible reflex mechanism acting through efferent vagal or sympathetic pathways in the observed response of coronary blood flow; absence of reflex effects due to changes in haemodynamic variables was expected, since such changes were prevented in the present investigation.

Given that L-NAME has been shown to prevent the formation of EDRF (Henderson, 1991), the results obtained after the administration of L-NAME, with or without the previous blockade of coronary cholinergic and adrenergic receptors, indicated that the response of coronary vasodilatation to the bolus infusion of hypertonic saline solution involved the release of EDRF. This mechanism was confirmed in the two animals in which the bolus infusion of hypertonic saline solution was performed only after the administration of L-NAME. In the present study, L-NAME abolished the coronary vasodilatory response caused by hypertonic saline solution even after the infusion of sodium nitroprusside, which reversed the vasoconstrictor effect of L-NAME. It is possible that the increase in mean coronary blood flow after stopping the infusion of hypertonic saline solution was due to the initial increase in flow that occurred during the infusion: an increase in coronary blood flow has been shown to release EDRF which causes vasodilatation in other segments of the coronary system (Holtz, Forstermann, Pohl, Giesler & Bassenge, 1984). However, the increase in coronary blood flow that occurred during the bolus infusion of normal saline was of the same magnitude as that produced by the hypertonic saline solution, and yet with normal saline solution there was no increase in flow after the end of the infusion. This suggests a further effect of hypertonic saline solution on the release of EDRF.

Previous reports have shown that stimulation of sympathetic or vagus nerves results in changes in coronary blood flow which can lead to the release of EDRF (Broten *et al.* 1992; Parent, al-Obaidi & Lavallée, 1993). In the present investigation the cholinergic and adrenergic receptors did not appear to play a significant role in the hypertonic saline solution-induced increase in coronary blood flow. This was also confirmed as this response still occurred after giving cholinergic and adrenergic blockers and could then be blocked by L-NAME.

In conclusion, the present study has shown that the intracoronary bolus infusion of hypertonic saline solution in anaesthetized pigs caused a sustained coronary vasodilatation, the mechanisms of which involved EDRF, but not cholinergic and adrenergic receptors.

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