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Effects of homocysteine on acetylcholine- and adenosine-induced vasodilatation of pancreatic vascular bed in rats

*I. Quéré, [1]D. Hillaire-Buys, C. Brunschwig, J. Chapal, *C. Janbon, J.P. Blayac, P. Petit & M.M. Loubatières-Mariani

Laboratoire de Pharmacologie (UPRES EA 1677), Faculté de Médecine, Boulevard Henri IV, Montpellier and *Service de Médecine Interne, Hôpital Saint Eloi, Montpellier, France

1 Epidemiological and experimental data have shown that homocysteine may provoke vascular lesions and that moderate homocysteinaemia may constitute an independent risk factor for vascular disease. It is now documented that homocysteine damages human endothelial cells in culture, possibly by producing hydrogen peroxide in an oxygen-dependent reaction.

2 In this study, we have examined the direct effect of this sulphur amino acid on pancreatic vascular resistance. Experiments were performed on the vascular bed of the rat isolated pancreas perfused at constant pressure; thus, any change in pancreatic vascular resistance resulted in a change in the flow rate. D,L-Homocysteine perfused for one hour at three different concentrations (200 μM, 2 mM, 20 mM) did not induce any significant change in the flow rate *per se*. Homocysteine infusion for 30 min at a concentration of 200 μM or 2 mM abolished the endothelium-dependent vasodilatation induced by acetylcholine (0.05 μM), but did not modify adenosine (1.5 μM)-induced vasodilatation.

3 The effect of D,L-homocysteine (200 μM or 2 mM) cannot be ascribed to a direct antimuscarinic effect since 30 min pretreatment of rat ileum with these concentrations did not significantly change the contractile effect of increasing concentrations of acetylcholine (0.015–15 μM).

4 Preincubation of human umbilical vein endothelial cells with D,L-homocysteine (0.2–5.0 mM) had no significant effect on overall cell number or viability during 18 h of incubation; the endothelial cells exposed to concentrations up to 5 mM exhibited a spindle-shaped, whirled pattern. This pattern was reversed 48 h after the removal of homocysteine. A cytotoxic effect was seen after 18 h incubation in 10 mM D,L-homocysteine.

5 In conclusion, an acute infusion of homocysteine altered acetylcholine endothelium-induced vasodilatation, whereas the adenosine vasodilatator effect was insensitive to the deleterious action of homocysteine *in vitro*.

Keywords: Homocysteine; thiol; vasodilatation; acetylcholine; adenosine; pancreatic vascular bed; endothelial cells

Introduction

Homocysteine, a thiol-containing amino acid, is an intermediate metabolite of methionine. Elevated levels of biological homocysteine are an independent risk factor for atherosclerosis (Clarke *et al*., 1991; Stampfer *et al*., 1992) and are associated with coronary and cerebrovascular arterial thrombosis (Coull *et al*., 1990). This fact is an attractive area of clinical research as evidenced by the number of recent publications on this topic (Boers *et al*., 1995; Dalery *et al*., 1995; den Heijer *et al*., 1995; 1996; Glueck *et al*., 1995; Landgren *et al*., 1995; Nygard *et al*., 1995; Perry *et al*., 1995; Selhub *et al*., 1995). In patients with homocystinuria, enzymatic deficiencies lead to elevated levels of plasma homocysteine, ranging from 100 to 500 μM (Malinow, 1994). This rare genetic disorder is associated with severe and premature arteriosclerosis (Mudd *et al*., 1964) and endothelial dysfunction (Celermajer *et al*., 1993). Oxidation products of homocysteine in plasma and cytosol may contribute to its vascular pathogenicity (Mc Cully, 1971; Kang *et al*., 1986). Homocysteine in blood, plasma or serum refers to total homocysteine and is the sum of homocysteine and oxidized products, whether free or bound to protein. The toxic effect of homocysteine on endothelium was first suggested in baboons (Harker *et al*., 1974; 1983) and subsequently confirmed (Wall *et al*., 1980; De Groot *et al*., 1983) and attributed to H_2O_2 generated by the way of the SH groups (Heinecke *et al*., 1987). Direct toxic effects of homocysteine thiolactone on endothelial cells have also been demonstrated (Mc Cully & Wilson, 1975). Recently, it has been found that the induction of hyperhomocysteinemia in minipigs induces hypertension and histological lesions of the vascular wall, characterized by endothelial and smooth muscle cell hypertrophy (Rolland *et al*., 1995). Furthermore, in non-human primates, diet-induced hyperhomocysteinemia lead to a diminished *ex vivo* vasodilatation induced by acetylcholine and nitroprusside (Lentz *et al*., 1996). Previous studies have described the endothelium-dependent relaxant properties of acetylcholine in resistance vessels (Owen & Bevan, 1985; Myers *et al*., 1989). *In vivo* and *in vitro* studies, with NO synthase inhibitors, have clearly demonstrated the key role of NO related substances in acetylcholine-induced vasodilatation in various species including rats, rabbits as well as primates (Sellke *et al*., 1990; Moncada *et al*., 1991), despite the fact that some of the NO inhibitors can act, at least in part, by a direct antagonism of muscarinic receptors (Buxton *et al*., 1993).

In order to investigate the direct influence of elevated levels of homocysteine on vascular resistance, we compared the effects of acute homocysteine infusion on the vasodilatation elicited by acetylcholine (an endothelium-dependent vasodilatator) and by adenosine, known to induce endothelium-independent relaxation. For that purpose, the experiments were performed on the pancreatic vascular bed of normal rats, which contains a network of arteries, veins and capillaries and in which endothelial cells were intact. This preparation has been shown to express cholinoceptors which mediate vascular relaxation (Bertrand *et al*., 1989). In the pancreatic vascular bed, purinoceptor-induced vasodilatation occurs via both P1 (A_2)-receptors for adenosine (Soulaymani *et al*., 1985) and P2

[1]Author for correspondence at: Laboratoire de Pharmacologie, Faculté de Médecine, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier Cedex 01, France.

(P2Y) receptors for ATP or ADP (Hillaire-Buys *et al*., 1991). In order to test the potential antagonism by homocysteine of muscarinic receptors, we performed experiments in rat ileum preparations. We also evaluated the effect of homocysteine on endothelial cell viability.

Methods

Rat pancreatic vascular bed

The experiments were carried out on the isolated perfused pancreas of the rat, according to the technique described previously (Loubatières *et al*., 1969). Male Wistar rats weighing 300–350 g received 60 mg kg^{-1} sodium pentobarbitone by i.p. injection. The pancreas was completely isolated from all neighbouring tissues and organs; it was perfused through its own arterial system with a Krebs-Ringer bicarbonate buffer (composition in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5) containing bovine serum albumin (2 g l^{-1}) and 5 mM glucose. A mixture of O_2 (95%) and CO_2 (5%) was bubbled at atmospheric pressure. The pH of the solution was 7.35. The preparation was maintained at 37.5°C. Each organ was perfused at a constant pressure (in the range of 40–50 cm H_2O), selected so as to obtain a flow rate of 2.5 ml min^{-1} at the start of the experiment. Any change in pancreatic vascular resistance due to drug aministration resulted in a change in the flow rate; the latter was measured by collecting each sample in a graduated tube for 1 min.

We carried out a kinetic study for 120 min, which was divided into four periods: (1) a 45 min equilibration period (0–45 min of organ perfusion) in which basal flow rates were measured twice, at 30 and 45 min before any drug administration; (2) the next 30 min (45–75 min of organ perfusion) in which D,L-homocysteine was either added or not (control); (3) in the third period (75–105 min) the vasodilator substance (acetylcholine or adenosine) was infused in the absence of any other drug (control experiments) or in the presence of the sulphur amino acid; (4) the final period (105–120 min) in which the flow rate was measured 5 and 15 min after the end of drug infusion, allowed us to determine the reversibility of flow rate changes.

The graphs represent the kinetics of flow rate. The flow rate was measured every min during the first 5 min, then 10, 15, 20 and 30 min after the addition of homocysteine and the vasodilator substance.

Isolated ileum preparation

Male Wistar rats (300–350 g) were anaesthetized with sodium pentobarbitone. A 2 cm segment of the ileum was removed and mounted on two supports. The upper end was attached to a Sanborn strain gauge and the lower to a stable mount. The preparation was vertically suspended in a 50 ml isolated organ bath filled with Krebs-Ringer bicarbonate buffer (composition, as above) containing 5 mM glucose maintained at 36±1°C and gassed with a mixture of O_2 (95%) and CO_2 (5%) at atmospheric pressure. After an equilibration period of 30 min, contractile responses were recorded isotonically with a transducer and a polygraph Sanborn 150 (Sanborn Company, Wahtham Mass, U.S.A.).

Cumulative concentration-contractile response curves for acetylcholine in the absence and presence of homocysteine were determined at 15 min intervals. To examine the effect of homocysteine on acetylcholine responses, the ileum segments were exposed to the amino acid for 30 min before the cumulative addition of the agonist. Each dose of acetylcholine was allowed to produce its full effect (15–30 s contact) before the concentration of the drug in the bath was increased in geometric progression of ratio two. The maximal contractile effect (E_{max}) was considered to be obtained when two successive increases in acetylcholine concentration gave the same contractile effect. Each experiment was separated from the other by at least two washes, in order to obtain the same baseline. Responses from each experiment were normalized as % of the maximal contractile effect of acetylcholine. Subsequent concentration-response curves were drawn and the EC_{50} values (the concentration producing a 50% maximal contractile effect) were determined for both groups by use of the Hill transformation: log E/log (E_{max} – E) versus log concentration. To examine the effect of homocysteine on acetylcholine responses, the ileum segments were exposed to the amino acid for 30 min before cumulative addition of the agonist.

Cell culture techniques and homocysteine treatment of cultured cells

Adult bovine aortic endothelial (ABAE) and human umbilical vein endothelial cells (HUVEC) were used during the first passage and cultured in 80% M199 and 20% foetal calf serum, antibiotics and amphotericin as described (Jaffe *et al*., 1973). Cells were plated in 24-well trays. D,L-Homocysteine was dissolved in serum-free medium and sterile filtered at the desired concentrations (0.20–10 mM). Incubations were performed on cell monolayers at 1 to 2 days preconfluence in the tissue culture incubator. Cell viability was assessed by use of the neutral red assay. (Shao-Zeng *et al*., 1990). Neutral red was dissolved in phenol free Hanks buffer (final concentration 0.66 mg l^{-1}) at 37°C and centrifuged at 2500 r.p.m. for 5 min. Cells were incubated with neutral red for 2 h at 37°C in the incubator and then rapidly washed in 1% formaldehyde and 1% $CaCl_2$. Neutral red incorporated in lysosomes was then diluted for 30 min in 1% acetic acid and 50% ethanol solution at room temperature. Quantification of incorporated neutral red was made by spectrophotometry (Titertek, Multiskan) at 540 nm.

Statistical analysis

The results are expressed as means±s.e.mean. Analysis of variance was applied, followed by the multiple comparison test of Neumann-Keuls (Zar, 1974). P values less than 0.05 were considered statistically significant.

Drugs used

Acetylcholine chloride, adenosine, 8-phenyl-theophylline, D,L-homocysteine were from Sigma Chemical Inc. (St. Louis, MO, U.S.A.). Drug solutions were freshly prepared and immediately used for each experiment. 8-Phenyl-theophylline was dissolved in sodium hydroxide in equimolar concentrations.

Results

Effect of homocysteine on pancreatic flow rate

In control experiments performed in the presence of glucose 5 mM alone, the vascular flow rate was not significantly modified during the entire 120 min of the experiment (Table 1). D,L-Homocysteine at the three concentrations used (200 μM, 2 mM, 20 mM) did not significantly modify the pancreatic flow rate during the one hour infusion period (Table 1). Although not significant, homocysteine tended to induce a vasodilatator effect, especially when the concentration of 2 mM was used.

Acetylcholine (0.05 μM) elicited an immediate vasodilatation which reached a maximum of +12.8% at time 95 min ($P<0.001$) and lasted throughout the infusion of the muscarinic agonist. Fifteen min after the end of the acetylcholine infusion, the pancreatic flow rate was again at the baseline (Figure 1). Homocysteine, at a concentration of 2 mM infused 30 min before and throughout the acetylcholine infusion, completely suppressed the vasodilatator effect of the latter (at 95 min, pancreatic flow rate was 2.46±0.08 ml min^{-1} for 2 mM homocysteine plus acetylcholine versus 2.81±0.05 ml min^{-1} for acetylcholine alone,

Table 1 Effects of the one hour infusion of homocysteine (from 45 min to 105 min) on pancreatic flow rate

	Time (min)									
	30	*40*	*45*	*50*	*60*	*75*	*80*	*90*	*105*	*120*
Glucose 5 mM alone	2.51	2.49	2.48	2.49	2.48	2.50	2.42	2.40	2.35	2.32
($n=4$)	±0.01	±0.02	±0.02	±0.03	±0.02	±0.04	±0.02	±0.04	±0.05	±0.04
Homocysteine 200 μM	2.47	2.50	2.50	2.54	2.44	2.54	2.49	2.43	2.49	2.22
($n=6$)	±0.02	±0.01	±0.02	±0.02	±0.04	±0.07	±0.05	±0.06	±0.05	±0.09
Homocysteine 2 mM	2.48	2.51	2.48	2.61	2.57	2.52	2.65	2.65	2.57	2.35
($n=6$)	±0.03	±0.04	±0.02	±0.04	±0.07	±0.08	±0.10	±0.10	±0.08	±0.09
Homocysteine 20 mM	2.47	2.47	2.47	2.59	2.56	2.51	2.58	2.58	2.53	2.46
($n=6$)	±0.02	±0.02	±0.02	±0.08	±0.10	±0.15	±0.09	±0.07	±0.08	±0.04

Results are expressed in absolute values (ml min^{-1}); n represents the number of experiments for each group.

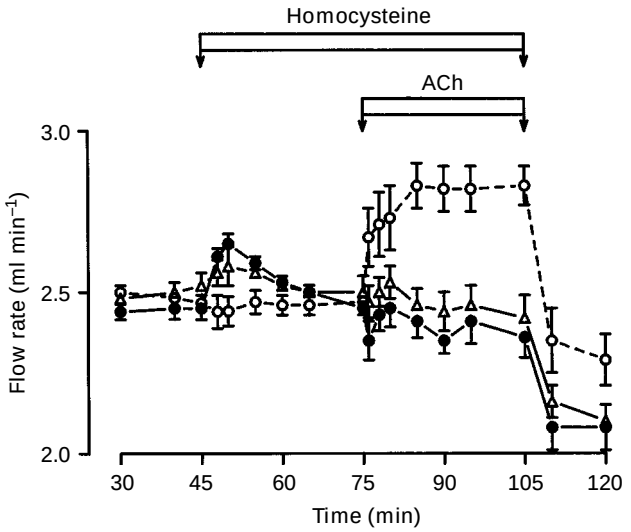

Figure 1 Effect of homocysteine on the acetylcholine (ACh)-induced vasodilatation of rat pancreatic vascular bed: (○) acetylcholine 0.05 μM alone; (●) acetylcholine 0.05 μM and homocysteine 2 mM, (△) acetylcholine 0.05 μM and homocysteine 200 μM. Each point represents the mean of five experiments; vertical lines show s.e. mean.

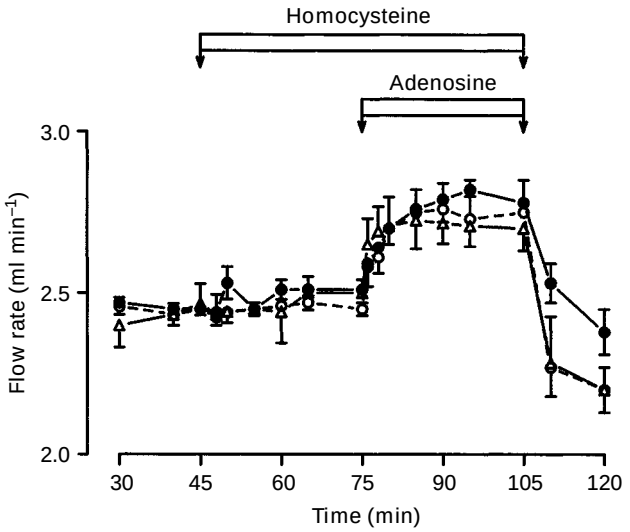

Figure 2 Effect of homocysteine on the adenosine-induced vasodilatation of rat pancreatic vascular bed: (○) adenosine 2 μM alone; (●) adenosine 2 μM and homocysteine 2 mM, (△) adenosine 2 μM and homocysteine 200 μM. Each point represents the mean of six experiments; vertical lines show s.e.mean.

$P<0.001$). A ten fold lower concentration of homocysteine was still able to counteract the vasodilatator effect of 0.05 μM acetylcholine (Figure 1).

The infusion of adenosine (2 μM) induced an immediate and long-lasting increase in the flow rate, reaching a maximum of +12.6% at time 90 min ($P<0.001$) (Figure 2). After the adenosine infusion had stopped, the pancreatic flow rate returned quickly to basal values. Homocysteine (200 μM), without significant effect *per se* during the first 30 min infusion, did not significantly change the vasodilatator effect induced by the nucleoside at any time (maximal effect of adenosine being respectively 2.73±0.09 ml min^{-1} for control experiments and 2.76±0.04 ml min^{-1} for homocysteine plus adenosine versus adenosine alone, $P>0.05$) (Figure 2). A ten fold higher concentration of homocysteine (2 mM) was also unable to modify the vasodilatator effect of the nucleoside (Figure 2). In either the absence or presence of 200 μM homocysteine, adenosine-induced vasodilatation was abolished by the purinoceptor antagonist 8-phenyl-theophylline (5 μM) (data not shown).

Effect of homocysteine on the contractile response of rat ileal smooth muscle

Cumulative concentration-response relationships for acetylcholine-induced contraction in ileum smooth muscle were determined by addition of acetylcholine (0.015–15 μM) in successive increments doubling from a given concentration to the following one. To determine whether the contractile response of acetylcholine was modified by homocysteine, cumulative additions of acetylcholine were also performed in the presence of homocysteine (200 μM or 2 mM), this amino acid being added 30 min before the acetylcholine. Addition of the sulphur amino acid to the incubation buffer did not alter the baseline tone. The effect of 200 μM homocysteine on the cumulative contractile responses of acetylcholine are shown in Figure 3. None of the two concentrations of homocysteine (200 μM or 2 mM) modified contractile effect of acetylcholine (Figure 4a and b). The concentrations of acetylcholine that produced half-maximal contraction were not significantly different in the absence and presence of homocysteine ($P=0.3$).

Effects of homocysteine on endothelial cultured cells

Preincubation of human umbilical vein endothelial cells with D,L-homocysteine (0.2–5.0 mM) for 18 h had no significant effect on overall cell number and phenotype (Figure 5a). Cell viability assessed by neutral red incorporation in response to 0.2 to 5.0 mM homocysteine differed from controls by 5% which is the method reproducibility (Figure 5c). After 24 hours of incubation, endothelial cells exposed to homocysteine, at concentrations ranging from 5.0 to 7.5 mM, exhibited a spindle-shaped, whirled pattern compared to the typical cobblestone appearance of control cell monolayers (Figure 5b). This pattern was reversible as 48 h after removal of homocysteine, the cell viability was identical to that of control. With 10 mM homocysteine, a toxic and irreversible effect was found after 18 h of incubation (Figure 5d).

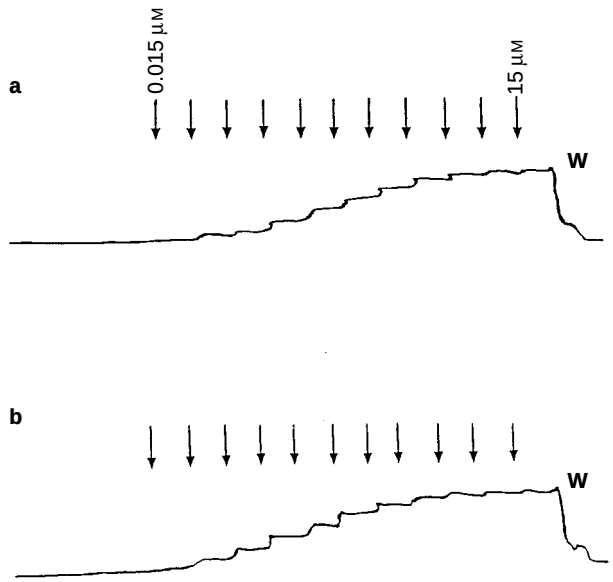

Figure 3 Typical records showing the contractile responses of a rat ileum segment to cumulative application of acetylcholine (0.015–15 μM). Arrows indicate the concentrations of acetylcholine added in a geometric progression of a ratio of two. (a) Control responses were obtained with increasing acetylcholine concentrations. (b) Homocysteine 200 μM was added 30 min before and during and the responses to acetylcholine were obtained.

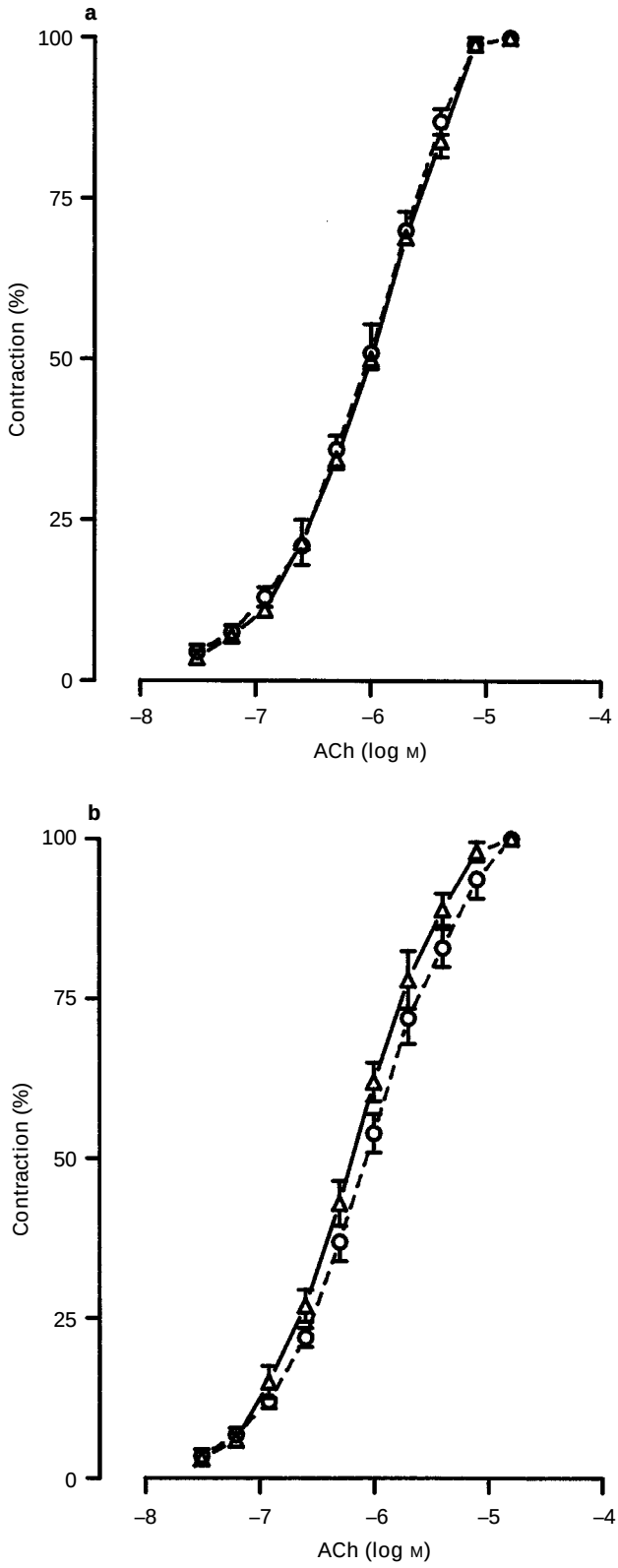

Figure 4 Dose-dependent response of rat ileum segments to acetylcholine (ACh). (a) (○) ACh alone, (△) ACh plus homocysteine 2 mM. (b) (○) ACh alone, (△) ACh plus homocysteine 200 μM. Each curve was drawn from the results obtained in four experiments. Ordinate scales show contractions expressed as a percentage of the maximal contractile effect. Vertical lines show s.e.mean.

Discussion

The present data demonstrated that an acute infusion of homocysteine completely suppressed acetylcholine-induced relaxation of the pancreatic vascular bed whereas the vasodilatation induced by adenosine remained unmodified. This vascular effect was observed with homocysteine concentrations in the range of those clinically observed in genetic disorders (Malinow, 1994). Stimulation of endothelial muscarinic receptors in most vascular beds produces smooth muscle relaxation as a result of the release of up to three relaxing agents: endothelium-derived relaxing factor (EDRF), now well identified as nitric oxide (NO), or related compounds such as nitrosothiols, prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF) (Rubanyi, 1991). This latter factor is neither a prostanoid (Feletou & Vanhoutte, 1988) nor an activator of guanylyl cyclase activity, but a smooth muscle hyperpolarizing factor (Chen *et al*., 1988; Taylor & Weston, 1988). In the rat perfused mesenteric arterial bed, it has been shown that both EDHF and endothelium-derived nitric oxide (EDNO) were released by the endothelium in response to acetylcholine (Adeagbo & Triggle, 1993). The EDHF-induced hyperpolarization has been shown to be mediated by activation of the sodium-potassium ATPase (Feletou & Vanhoutte, 1988) and/or by a direct opening of ATP-dependent or calcium-dependent potassium channels (Standen *et al*., 1989; Adeagbo & Malik, 1990) and/or through inhibition of apamin sensitive K^+ channels (Garcia-Pascual *et al*., 1995). NO- and PGI_2-dependent relaxant effects of acetylcholine may be secondary to the activation of muscarinic M_2 receptors (Rubanyi, 1991), whereas the subtype of muscarinic receptor involved in EDHF release seems to be more controversial, M_1 or M_3 subtypes according to the preparation (Rubanyi, 1991; Hammarström *et al*., 1995). The abolition of acetylcholine-induced relaxation by homocysteine in our preparation seems not to be related to a direct antagonist effect of homocysteine on muscarinic receptors, since this amino acid does not alter the contractile effect of acetylcholine on intestinal smooth muscle, an effect mediated through the activation of M_2 or M_3 receptors (Zhang *et al*., 1991; Thomas *et al*., 1993). With regard to the involvement of NO in vascular relaxation, it is well documented that this compound plays a role in most species and types of preparation (Moncada *et al*., 1991). Since L-NAME evoked a potent vasoconstriction *per se* in the pancreatic vascular bed, it has been suggested that basal release of NO may be responsible for maintaining vasodilatator tone in this preparation (Saiag *et al*., 1996). The fact that homocysteine

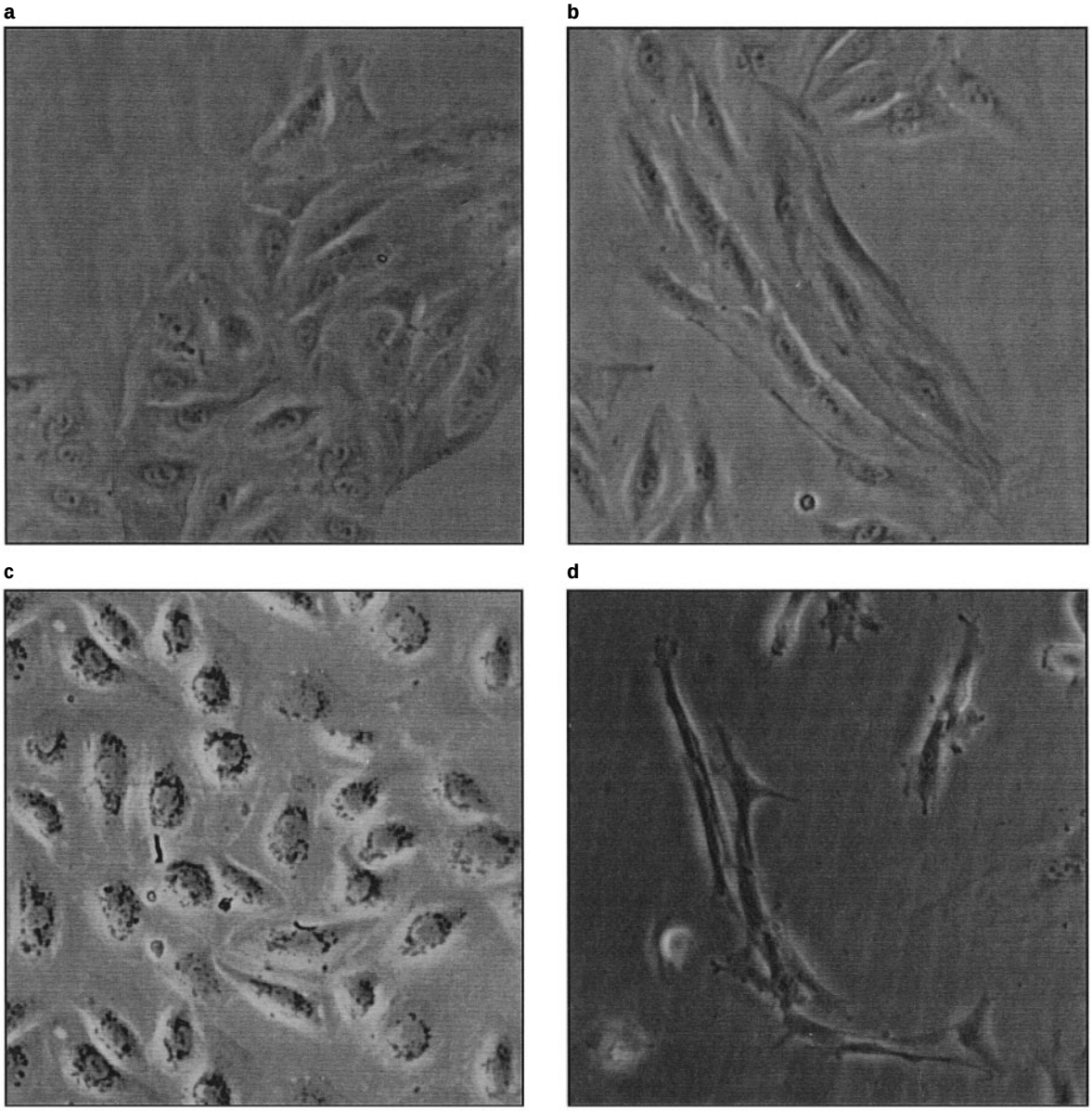

Figure 5 Effect of D,L-homocysteine on endothelial cell morphology and viability. Preconfluent endothelial cells were incubated for 18 h with D,L-homocysteine at concentrations of 2 mM (a). After 24 h of incubation with 7.5 mM homocysteine, cells exhibited an elongated shape (b). Lysosomial neutral red inclusion was used as a control of cell viability after incubation with homocysteine 2 mM for 18 h (c). A cytotoxic effect was seen after 24 h of incubation with 10 mM homocysteine (d).

does not decrease the basal pancreatic flow rate and, even, in most cases tends to induce a slight relaxation, although not significant, might be ascribed to the ability of homocysteine to generate vasodilator S-nitroso-thiols from the basal NO release during a one hour exposure. In fact, it has been shown that prolonged (more than three hours) exposure of cultured endothelial cells to homocysteine results in impaired endothelium-derived relaxing factor (EDRF) responses whereas brief (less than 15 min) exposure stimulated these cells to secrete EDRF, resulting in the formation of S-nitroso-thiols (Stamler *et al*., 1993). Moncada and his colleagues (1991) have demonstrated that different concentrations of L-cysteine can also modify, in a biphasic way, NO and S-nitrosocysteine-induced relaxation of endothelium-intact aortic ring preparations (Feelish *et al*., 1994). Recently, it has been proposed that the formation of S-nitroso-thiols from homocysteine plus NO can attenuate the adverse vascular effects of homocysteine. When exposure to this amino acid was increased, the formation of S-nitroso-thiols may be outmatched by the deleterious effects of the oxygen reactive species formed (Upchurch *et al*., 1996). The total suppression of the relaxant effect of acetylcholine suggests that an infusion of homocysteine, at a concentration observed in human genetic diseases, abolishes the relaxation dependent on the release of endothelial vasodilator factors. This alteration in acetylcholine-induced vascular relaxation may be related to a functional impairment of endothelial cells rather than to a cytotoxic effect, since concentrations of homocysteine up to 10 mM were required to decrease the viability of endothelial cells. The precise level at which homocysteine has an effect (i.e. coupling between receptor, NO synthase or subsequent steps) remains to be defined.

In contrast to the previous findings, the present study showed that the adenosine vasodilator effect is preserved in the presence of high levels of homocysteine. This adenosine effect was abolished by the P1 purinoceptor antagonist 8-phenyltheophylline (Fredholm *et al*., 1994). The adenosine vascular effect is classically ascribed to an endothelium-independent process involving adenylyl cyclase and/or K_{ATP} channel activation (Fredholm *et al*., 1994). However, in the mesenteric arterial bed of the rat, the vascular relaxant effect of adenosine has recently been shown to be, at least in part, dependent on

the presence of the endothelium (Hiley *et al*., 1995). The discrepancy found in the latter study between the effect of L-NAME and that of endothelium removal (no effect for the former and decreased vasodilator effect to adenosine for the latter) may suggest the possible involvement of EDHF release in adenosine-induced vasodilatation. In our conditions, the fact that homocysteine did not modify the effect of adenosine indicates either a total endothelium-independence of the vasodilatator mechanisms underlying adenosine relaxation, or profound discrepancies in the EDHF release elicited by adenosine and acetylcholine.

Homocysteine is a product of the transmethylation pathway and is formed from S-adenosylhomocysteine, the cleavage of which is catalysed by adenosylhomocysteine hydrolase to give homocysteine plus adenosine. The effects of this sulphur amino acid in thrombosis can be related to its proaggregatory properties (Mc Cully & Carvalho, 1987), despite the fact that the underlying mechanisms remain to be elucidated. Furthermore, incubation with millimolar concentrations of homocysteine for six days increases the growth of vascular smooth cells in culture (Tsai *et al*., 1994), a factor known to play a key role in the early states of atherosclerosis (Ross, 1993). In addition to the alteration of the NO pathway by homocysteine, inhibition of PGI_2 in human endothelial cells by homocysteine has been demonstrated recently (Quéré *et al*., 1995); this may increase the risk of vascular damage induced by the sulphur amino acid, if the role of arachidonic acid products in the regulation of blood flow and platelet activation is considered (Radomski *et al*., 1987). Thus, homocysteine seems to be at the parting of the ways of the regulating mechanisms underlying platelet aggregation, smooth muscle cell proliferative state and vasorelaxation. On the other hand, the fact that dipyridamole, a well known inhibitor of adenosine uptake, prevented the preatherosclerotic lesions induced by homocysteine (Harker *et al*., 1976) suggests that adenosine might counteract the harmful effects of increased levels of the thiol-containing amino acid. Furthermore, it has been recently shown that adenosine treatment inhibits the growth of smooth muscle cells in culture, particularly those originating from rats with streptozotocin-induced diabetes, a well known state which accelerates atherosclerosis (Parès-Herbuté *et al*., 1996). Finally, this study demonstrates that the adenosine vasodilator effect is insensitive to the deleterious action of homocysteine *in vitro*. The preservation of the adenosine effect might be related to the antioxidant properties of adenosine (Ramkumar *et al*., 1995). This hypothesis needs to be explored further. In conclusion, an acute infusion of homocysteine altered the endothelium dependent vasodilatation induced by acetylcholine, but did not modify the vascular relaxation to adenosine.

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