Effect of inhibition of nitric oxide synthesis on the uptake of LDL and fibrinogen by arterial walls and other organs of the rat

¹L.E. Cardona-Sanclemente & G.V.R. Born

Pathopharmacology Unit, The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ

1 The effects of administering 3 mg ml⁻¹ N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO), on the uptake of low density lipoprotein (LDL), fibrinogen and blood pressure were determined in conscious, unrestrained, cannulated normotensive and spontaneously hypertensive (SHR) Wistar rats.

2 The uptake of LDL and fibrinogen, labelled respectively with ¹²⁵I or ¹³¹I via the adduct tyramine cellobiose ([¹²⁵I]TC-LDL and [¹³¹I]TC-fibrinogen), were compared in aortic walls, heart, skeletal muscle, lung, liver, kidney and adrenal during the final 24 h of 6 days' administration of L-NAME in the drinking water.

3 In control normotensive rats, the systolic blood pressure did not change significantly over 6 days, while administration of L-NAME in normotensive rats increased the blood pressure progressively and significantly to about 170 mmHg over the same period.

4 In normotensive rats L-NAME increased significantly the uptake of both LDL and fibrinogen by aortic walls and heart, but not by muscle, lung, liver, kidney and adrenal.

5 The blood pressure in SHR was about 170 mmHg before administration of L-NAME and did not increase significantly after 6 days of treatment. In these rats the uptake of LDL or of fibrinogen was increased only in the heart but not in aortic walls nor in any of the other organs.

6 In normotensive rats the increase in blood pressure caused by inhibition of NO generation was associated with increases in the uptake of the atherogenic plasma proteins LDL and fibrinogen by the wall of the aorta and by the heart but not by the skeletal muscle, lung, liver, kidney and adrenal. The slightly higher blood pressure of SHR treated with L-NAME was not associated with increased uptake of LDL or fibrinogen by aorta nor by any organ except heart. Thus, while in normotensive rats the increase in blood pressure caused by inhibition of NO generation was associated with increase in the uptake of atherogenic plasma proteins LDL and fibrinogen by the wall of the aorta and by the heart, in SHR which showed uptakes similar to the normotensive animals, the non-significant increase in blood pressure induced by inhibition of NO generation was not associated with increased uptake of LDL or fibrinogen in any organ except the heart.

7 These results are consistent with effects, demonstrated earlier, of infusing the pressor agents noradrenaline, adrenaline and angiotensin II, which produce increases in uptake of LDL and fibrinogen by aortic wall, whereas this is not so in spontaneously hypertensive rats.

Keywords: N^G-nitro-L-arginine methyl ester (L-NAME); blood pressure; low density lipoprotein (LDL); fibrinogen; uptake; rat, normotensive and hypertensive

Introduction

Previous experiments have shown that infusions of noradrenaline or adrenaline into carotid arteries of anaesthetized rabbits to produce moderate increases in their local blood concentrations increase the uptake of atherogenic plasma proteins, low density lipoproteins (LDL) and fibrinogen by the artery walls (Shafi *et al.*, 1989; Cardona-Sanclemente & Born, 1992a). Furthermore, systemic infusions of adrenaline or angiotensin II into conscious unrestrained rats, producing slight to moderate rises in blood pressure, increase the uptake of LDL and fibrinogen by aortic walls (Cardona-Sanclemente & Born, 1992b; Cardona-Sanclemente *et al.*, 1994a). However, in spontaneously hypertensive rats of the same age in which the blood pressure was higher than in those infused with the pressor agents, the uptake of LDL and fibrinogen was the same as in the controls, and was also not increased by infusions of angiotensin II (Cardona-Sanclemente *et al.*, 1994b).

Thus the mechanism(s) responsible for the increased uptake of the atherogenic plasma proteins and, specifically,

the involvement of blood pressure is not clear. We now show that yet another means of increasing blood pressure, namely infusion of an inhibitor of endogenous nitric oxide (NO) formation, is also associated with increases in LDL and fibrinogen uptake by aortic walls in conscious, unrestrained normotensive but not in hypertensive (SHR) Wistar rats.

The formation of nitric oxide by the inducible synthase has been demonstrated in several tissues and cells (Moncada, 1992). We, therefore, took the opportunity to determine the effect of inhibition of NO formation on the uptake of LDL and fibrinogen by other organs.

Methods

Animals

Fourteen adult male Wistar rats (300 ± 14 g) and seven SHR (293 ± 5 g) were fed *ad libitum* on standard diet. The rats were kept in separate wire-bottomed cages in an air-conditioned animal house with a light period lasting from 08 h 00 min to 20 h 00 min.

¹ Author for correspondence.

L-NAME administration

A group of 7 Wistar and the 7 SHR rats received N^G-nitro-L-arginine methyl ester (L-NAME; Sigma Chemical Company, Dorset) in the drinking water for six days. The controls were 7 weight-matched Wistar rats drinking tap water only. L-NAME was dissolved in the drinking water at a concentration of 3.0 mg ml⁻¹. In preliminary experiments, this dose elicited the most reproducible increases in blood pressure.

Fibrinogen

Lyophilized human fibrinogen (KabiVitrum, Sweden) was dissolved in physiological saline to give a concentration of 100 mg ml⁻¹.

Low density lipoprotein preparation

Human blood was obtained by venepuncture from healthy students or laboratory personnel with lipid levels in the normal range. After mixing with 0.15 M EDTA, the blood was centrifuged at 8700 g for 30 min at 4°C. LDL (density 1.019–1.063 g ml⁻¹) was separated by floatation after sequential centrifugation at 105000 g for 24 h at 4°C on KBr solutions adjusted to a final density of 1.019 and 1.063 (Havel *et al.*, 1955; Koga *et al.*, 1969; Fidge & Poulis, 1978; Nagelkerke *et al.*, 1984; Cardona-Sanclemente *et al.*, 1988). The final LDL fraction was dialysed at 4°C against phosphate buffered saline containing 0.3 mM EDTA at pH 7.5 to remove excess salts. Protein content was measured by the BCA assay reagent (Pierce Chemical Co., U.S.A.).

Labelling and iodination of LDL and fibrinogen

An adduct of cellobiose and tyramine (TC) was radioiodinated with ¹²⁵I or ¹³¹I and attached covalently to LDL or fibrinogen respectively. The labelled TC remains *in situ* when the lipoprotein or fibrinogen undergo degradation, so that the radioactivity is a measure of the proteins which were present in the tissues (Pittman *et al.*, 1983). The labelled proteins were dialysed in cold phosphate-buffered saline pH 7.5 containing EDTA. The tyramine cellobiose-labelled LDL ([¹²⁵I]-TC-LDL) and fibrinogen ([¹³¹I]-TC-fibrinogen) had specific activities of 100–400 c.p.m. ng⁻¹ protein and free ¹²⁵I content of 1–4% for the LDL fraction, and of 50–150 c.p.m. ng⁻¹ protein and free ¹³¹I content of 1–3.5% for the fibrinogen preparation.

Surgical procedures

Rats were anaesthetized with intramuscular Hypnorm (Janssen) 0.3 ml kg⁻¹ and intraperitoneal methyl diazepam-one (Diazepam, Phoenix Pharm.) 2.5 mg kg⁻¹. An incision was made in the neck and the right common carotid exposed. A fine polythene cannula was tied into the carotid artery. The cannula, previously closed with a pin, was passed through a subcutaneous tunnel in the skin to exit dorsally at the back of the neck, and the ventral incision was closed by suture. After surgery each rat was housed individually and allowed to recovery overnight before beginning the experiment proper. The patency of the cannula was maintained by a daily injection of 0.2 ml hirudin (10 µg ml⁻¹). Five days after surgery the rats were anaesthetized with Hypnorm alone and the labelled proteins injected through the femoral vein. Twenty-four hours later the rats were anaesthetized and killed by exsanguination via the abdominal aorta. After puncturing the abdominal aorta for collecting blood and for washing out the cardiovascular system with saline, the whole aorta was exposed from the heart to the bifurcation and excised intact. Surrounding fat and loose connective tissue were removed under a stereomicroscope. The vessel was washed 5 times in ice-cold buffer and placed in preweighed tubes. Samples of other organs and tissues were excised and

their radioactivities the samples were dried at 60°C to constant weight. Results are expressed as ng apo B protein per mg dry weight. All procedures were approved by the Animal Care and Safety Committee and conformed to guidelines established by the Home Office.

Blood pressure measurements

The rats' blood pressure and heart rate were determined daily in the conscious, unrestrained animals by connecting the indwelling catheter in the carotid artery to a transducer coupled to a Multitrace 2 Recorder 5022 (Lectromed, U.K.).

Statistical analysis

The significance of differences among groups of animals was assessed by Student's two-tailed *t* test for unpaired samples. A *P* value of less than 0.05 was taken as significant. Results are shown as mean ± s.e.mean.

Results

When normotensive rats were given L-NAME in the drinking water at doubling concentrations from 0.75 to 3.0 mg ml⁻¹, the aortic uptake of LDL was significantly increased only by the highest concentration (Figure 1). Normotensive and hypertensive rats treated with L-NAME at this concentration appeared to remain as healthy as the control rats of the same age, although the body weights of the hypertensive rats decreased very slightly and not significantly compared to the normotensive rats, the body weights of which remained constant (Figure 2). The body weight of normotensive rats receiving L-NAME decreased by about 17% compared to control rats during the experiment.

Administration of L-NAME *ad libitum* in the drinking water at a concentration of 3.0 mg ml⁻¹ decreased the oral water intake by 27% (11.3 ml day⁻¹) in normotensive rats and by 16% (13.0 ml day⁻¹) in SHR compared with the control group (15.5 ml day⁻¹). Because of these decreases in water consumption, the daily doses of L-NAME were

Figure 1 Dose-response effect of N^G-nitro-L-arginine methyl ester (L-NAME) on aortic uptake of human low density lipoprotein (LDL) by normotensive rats. L-NAME was administered for 6 days in the drinking water at concentrations of 0.75 (*n* = 5), 1.5 (*n* = 6) and 3.0 (*n* = 6) mg ml⁻¹, shown respectively by the increasingly shaded columns from left to right. The open column represents the controls, i.e. no L-NAME in the drinking water. The values represent means ± s.e.mean: **P* < 0.05; ***P* < 0.01.

33.9 mg day⁻¹ in the normotensive and 39.0 mg day⁻¹ in the spontaneously hypertensive rats.

During 6 days administration of L-NAME to normotensive rats the systolic blood pressure increased progressively from 131 ± 8 to 173 ± 9 mmHg (mean ± s.e.mean), i.e. an increase of 32% compared to controls (Figure 3). In SHR treated with L-NAME the systolic blood pressure began higher than in the normotensive rats receiving L-NAME and rose during the first three days from 170 ± 3 to 198 ± 8 mmHg followed, by a decrease almost to the initial value at 185 ± 10 mmHg.

Figure 2 Changes in body weight of conscious, unrestrained normotensive and hypertensive Wistar rats during administration of N^G-nitro-L-arginine methyl ester (L-NAME) in the drinking water for 6 days. Control, (□), *n* = 6; normotensive rats + L-NAME, (●), *n* = 7; and SHR + L-NAME, (■), *n* = 7. L-NAME was administered in the water at a concentration of 3 mg ml⁻¹. The values represent means ± s.e.mean.

Figure 3 Effect of administration of N^G-nitro-L-arginine methyl ester (L-NAME) in the drinking water for 6 days on diastolic blood pressure of conscious, unrestrained normotensive and hypertensive Wistar rats. Control, (□), *n* = 6; normotensive rats + L-NAME, (●), *n* = 7; and SHR + L-NAME, (■), *n* = 7. The values represent means ± s.e.mean: **P* < 0.05; ***P* < 0.01.

In the normotensive rats administration of L-NAME for 6 days produced significant increases in the uptake by the aorta of both LDL and fibrinogen over the final 24 h period (Figures 4 and 5). By contrast, similar administration of L-NAME to SHR rats did not increase the uptake by aorta of either LDL or fibrinogen over that in the control rats (Figures 4 and 5).

In control rats the amounts of LDL taken up per mg dry weight increased in the order aorta < heart < muscle < lung < kidney < adrenal < liver, the hepatic uptake being about 15 times greater than the aortic. For fibrinogen the uptake was aorta ≤ heart < adrenal ≤ lung ≤ muscle < liver ≤ kidney, the hepatic and renal uptakes being about 18 times greater than the aortic.

Administration of L-NAME brought about highly significant increases in uptake of both LDL and fibrinogen in the hearts of normotensive rats as well as significant increases in the hearts of SHR compared to normotensive controls.

Figure 4 Aortic uptake of human low density lipoprotein (LDL) in the final 24 h of six days administration of N^G-nitro-L-arginine methyl ester (L-NAME) in the drinking water. Control, open column, *n* = 6; control rats + L-NAME, solid column, *n* = 7; and SHR + L-NAME, stippled column, *n* = 7. The values represent means ± s.e.mean: **P* < 0.05; ***P* < 0.01.

Figure 5 Aortic uptake of human fibrinogen in the final 24 h of six days administration of drinking water (control, open column, *n* = 6; normotensive rats (control + L-NAME, solid column, *n* = 7); and SHR (SHR + L-NAME, stippled column, *n* = 7). The values represent means ± s.e.mean: **P* < 0.05; ***P* < 0.01.

Figure 6 Uptake of human low density lipoprotein (LDL) by different organs in the final 24 h of six days administration of N^G -nitro-L-arginine methyl ester (L-NAME) in the drinking water. Control rats, open column, $n = 6$; control rats receiving L-NAME solid column, $n = 7$; and SHR receiving L-NAME, stippled column, $n = 7$. The values represent means $\pm$ s.e.mean: * $P < 0.05$; ** $P < 0.01$.

Figure 7 Uptake of human fibrinogen into different organs in the final 24 h of six days administration of N^G -nitro-L-arginine methyl ester (L-NAME) in the drinking water. Control rats, open column, $n = 6$; control rats receiving L-NAME, solid column, $n = 7$; and SHR receiving L-NAME, stippled column, $n = 7$. The values represent means $\pm$ s.e.mean: * $P < 0.05$; ** $P < 0.01$.

However, L-NAME did not increase the uptake of either protein in any of the other organs, viz. skeletal muscle, lung, kidney, liver and adrenal (Figures 6 and 7).

Discussion

The technique of labelling LDL and fibrinogen with radioiodine via tyramine cellobiose ($[^{125}\text{I}]\text{-TC-LDL}$ and $[^{131}\text{I}]\text{-TC-fibrinogen}$ respectively) means that the radioactivities measured in aortic walls and other organs do not distinguish between labelled breakdown products accumulating over the 24 h periods during which the labelled LDL and fibrinogen were circulating, and whatever amounts of intact labelled LDL and fibrinogen were present at the time of measurement. We are aware that the parameter we term uptake is

predominantly a measure of such accumulation. Experiments now underway will also quantify the amounts of labelled unmetabolized LDL in the wall.

The present results show that in conscious, unrestrained, normotensive rats the uptake of LDL and fibrinogen by the aortic walls is increased by administration of L-NAME. L-NAME inhibits the formation of the potent vasodilator substance nitric oxide (NO) from L-arginine by endothelial cells (Moncada *et al.*, 1988). Our rats showed the expected progressive rise in blood pressure (Rees *et al.*, 1990), so that moderate hypertension accompanied the increased uptake of the atherogenic plasma proteins by aortic walls. In this respect, therefore, our findings with L-NAME resemble those previously obtained with other pressor agents viz. noradrenaline, adrenaline and angiotensin II. Indeed, this previously observed association of uptake and blood pressure increases was a major reason for determining the effect of L-NAME as yet another means of raising blood pressure.

At first sight, therefore, the latest results would support some direct connection between the two effects. Such a connection would also plausibly emerge from observations showing that the labelling of vascular endothelial cell vesicles with ferritin is increased with increasing intraluminal pressure (Moffitt *et al.*, 1992). However, some of our own findings are inconsistent with such a conclusion. Thus, angiotensin II infused at a concentration sufficiently low as to cause only a very small and temporary rise in blood pressure still produced highly significant increases in LDL and fibrinogen uptake (Cardona-Sanclemente *et al.*, 1994b). Even more striking was the absence of any increase in uptake in age-matched spontaneously hypertensive rats in which the blood pressure was higher than that produced by any of the administered pressor agents, although still well within the range occurring in clinical hypertension. For this there are, of course, several possible explanations. SHR begin to be hypertensive soon after birth, so that three-months' old animals may have developed some kind of adaptation to the mechanism(s) responsible for the uptake increases produced by administered pressor agents after five or six days. The striking lack of the uptake-increasing effect of L-NAME, as of angiotensin II, in the SHR also suggests the development of an adaptive process in these rats. Alternatively, although both control and spontaneously hypertensive rats were Wistars, there may have been genetic factors to account for the difference.

The determination of uptake by internal organs revealed interesting differences. Thus, the uptake of LDL was greatest in the liver, as expected from the normal metabolic routing of most circulating LDL via the hepatic high affinity receptors. The similarly large uptake by the adrenals was less expected because, in the rat, most of the cholesterol for steroid production comes from high density lipoprotein (HDL) (Gwynne & Strauss, 1982). LDL uptake was much less in the heart and even less in the aorta, presumably reflecting their comparative deficiency in high affinity receptors (Vasile *et al.*, 1983). If, therefore, LDL uptake by aorta and, perhaps, by heart was mostly by the non-receptor-mediated pathway underlying atherogenesis (Vasile *et al.*, 1983; Born, 1991), this would appear to be the mechanism potentiated by L-NAME and other pressor agents. This conclusion is supported by the fact that the uptake of fibrinogen, which is not known to involve any receptor mechanism, was increased by L-NAME only in the aorta and heart.

The large uptake of fibrinogen by liver and kidney was unexpected and appears to be a new observation (Hantgan *et al.*, 1994). The concentration of fibrinogen in the plasma is a major risk factor for coronary heart disease and stroke (Heinrich *et al.*, 1994; Wilhelmsen *et al.*, 1984). In relation to this, great importance attaches to factors which control this concentration, particularly the rates of biosynthesis of fibrinogen and of its removal from the circulation, and the uptake by liver and kidney presumably represents a major mechanism of fibrinogen removal.

The greatly increased uptake of LDL and fibrinogen brought about by L-NAME in the heart but in none of the other organs may be of great significance. This effect was so large that it was present also, although reduced, in the hearts of the SHR. What aorta and other arteries have in common with the heart and what differentiates them from other organs are the large, constantly repeated motions due to the heart beat. The results suggest, therefore, that reducing nitric oxide increases the effect of pulsatility on macromolecular flux into heart and arteries. In the heart, moreover, the increased uptake was so great as to make it unlikely to have occurred only in the coronary arteries but possibly in the myocardium as well.

Considering our experimental findings in relation to human atherosclerotic disease, it is nevertheless interesting that the risk of both coronary heart disease and stroke is increased more than additively when both LDL or fibrinogen and systolic or diastolic blood pressures are high (Wilhelmsen

et al., 1984; Heinrich *et al.*, 1994). If our work should after all point to some mechanistic connection between blood pressure and the accumulation of atherogenic plasma proteins in arterial walls they would provide, at least in principle, an explanation for these epidemiological facts.

Whether or not this is so, our findings indicate that nitric oxide limits the flux of the atherogenic plasma proteins into artery walls. In this, the effect of nitric oxide is similar to that of the net negative charge on arterial endothelium (Görög & Born, 1982; Ludlam *et al.*, 1991) although of course, through entirely different mechanisms. Our results indicate a novel anti-atherosclerotic effect of nitric oxide and so another factor in atherogenesis.

We wish to thank Dr Salvador Moncada, F.R.S., of the Wellcome Foundation and the Garfield Weston Foundation for generous support.

References

- BORN, G.V.R. (1991). Calcium and atherosclerosis. In *Calcium Antagonists and Atherosclerosis*. ed. Born, G.V.R., Triggle, D.J. & Poole-Wilson, P.A. pp. 5–6. London: Science Press.
- CARDONA-SANCLEMENTE, L.E. & BORN, G.V.R. (1992a). Adrenaline increases the uptake of low-density lipoproteins in carotid arteries of rabbits. *Atherosclerosis*, **96**, 215–218.
- CARDONA-SANCLEMENTE, L.E. & BORN, G.V.R. (1992b). Increased uptake of fibrinogen into arterial walls of unrestrained conscious rats caused by adrenaline and angiotensin II. *Br. J. Pharmacol.*, **107**, 317P.
- CARDONA-SANCLEMENTE, L.E., FEREZOU, J. & LUTTON, C. (1988). Cholesterol metabolism in the genetically hypercholesterolemic (RICO) rat. II. A study of plasma lipoproteins and effect of dietary cholesterol. *Bioch. Biophys. Acta*, **960**, 382–389.
- CARDONA-SANCLEMENTE, L.E., MEDINA, R. & BORN, G.V.R. (1994a). Effect of increasing concentrations of angiotensin II infused into unrestrained conscious rats on LDL and fibrinogen uptake by aorta. *Br. J. Pharmacol.*, **111**, 22P.
- CARDONA-SANCLEMENTE, L.E., MEDINA, R. & BORN, G.V.R. (1994b). Effect of increasing doses of angiotensin II infused into normal and hypertensive rats on low density lipoproteins and fibrinogen uptake by aortic walls. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 3285–3288.
- FIDGE, N.H. & POULIS, P. (1978). Metabolic heterogeneity in the formation of low density lipoproteins from very low density lipoproteins in rat: evidence of the independent production of a low density lipoprotein subfraction. *J. Lipid Res.*, **19**, 342–349.
- GROG, P. & BORN, G.V.R. (1982). Increased uptake of circulating low density lipoprotein and fibrinogen by arterial walls after removal of sialic acids from their endothelial surfaces. *Br. J. Exp. Pathol.*, **63**, 447–451.
- GWYNNE, J.T. & STRAUSS, J.F. (1982). The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroideogenic glands. *Endocrine Rev.*, **3**, 299–329.
- HANTGAN, R.R., FRANCIS, C.W. & MARDER, V.J. (1994). Fibrinogen structure and physiology. In *Haemostasis and Thrombosis: Basic Principles and Clinical Practice*. ed. Colman, R.W., Hirsh, J., Marder, V.J. & Salzman, E.W. 3rd edition, Ch. 14. Philadelphia: Lippincott.
- HAVEL, R.J., EDNER, H.A. & BRAGDON, J. (1955). The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.*, **34**, 1345–1353.
- HEINRICH, J., BALLEISEN, L., SCULTE, H., ASSMANN, G. & VAN DE LOO, J. (1994). Fibrinogen and Factor VII in the prediction of coronary risk: Results from the PROCAM study in healthy men. *Atheroscler. Thromb.*, **14**, 54–59.
- KOGA, S., HORWITZ, D.L. & SCANU, A.M. (1969). Isolation and properties of lipoproteins from normal rat serum. *J. Lipid Res.*, **10**, 577–588.
- LUDLAM, C., ARISAWA, A. & BORN, G.V.R. (1991). Increased uptake of low density lipoprotein after heparinase perfusion of carotid arteries in anaesthetised rabbits. *J. Physiol.*, **446**, 220P.
- MONCADA, S. (1992). The L-arginine: nitric oxide pathway. *Acta Physiol. Scand.*, **145**, 201–227.
- MONCADA, S., RADOMSKI, M.W. & PALMER, R.M.J. (1988). Endothelium-derived relaxing factor. *Biochem. Pharmacol.*, **37**, 2495–2501.
- MOFFITT, H., CLOUGH, G. & MICHEL, C.C. (1992). Effects of intraluminal pressures on the labelling of endothelial cell vesicles with native ferritin. *Int. J. Microcirc. Clin. Exp.*, **11**, 90.
- NAGELKERKE, J.F., HAVEKES, L., VAN HINSBERGH, V.W.M. & VAN BERKEL, T.J.C. (1984). In vivo and in vitro catabolism of native and biologically modified LDL. *FEBS Lett.*, **171**, 149–153.
- PITTMANN, R.C., CAREW, T.E., GLASS, C.K., GREEN, S.R., TAYLOR, C.A. & ATTIE, A.D. (1983). A radioinated, intracellularly trapped ligand for determining the sites of plasma protein degradation in vivo. *Biochem. J.*, **212**, 791–800.
- REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br. J. Pharmacol.*, **101**, 746–752.
- SHAFI, S., CUSACK, N.J. & BORN, G.V.R. (1989). Increased uptake of methylated low density lipoprotein induced by noradrenaline in carotid arteries of anaesthetised rabbits. *Proc. R. Soc.*, **235**, 289–298.
- VASILE, E., SIMIONESCU, M. & SIMIONESCU, N. (1983). Visualization of the binding, endocytosis, and transcytosis of low-density lipoproteins in the arterial endothelium in situ. *J. Cell Biol.*, **96**, 1677–1689.
- WILHELMSSEN, L., SVARDSUDD, K., KORSAN-BENGTSEN, K., LARSSON, B., WELIN, L. & TIBBLIN, G. (1984). Fibrinogen as a risk factor for stroke and myocardial infarction. *New Engl. J. Med.*, **311**, 501–505.

(Received August 8, 1994

Revised November 15, 1994

Accepted December 21, 1994)