JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1994, 45, 2, 259—269

B. MISZCZUK-JAMSKA, I. WYBRAŃSKA, J. HARTWICH, A. DEMBIŃSKA-KIEĆ, *R. J. GRYGLEWSKI

MODULATION OF LDL CATABOLISM BY SODIUM NITROPRUSSIDE AND PGE_2 IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

Department of Clinical Biochemistry Collegium Medicum Jagiellonian University, Cracow, Poland *Department of Pharmacology Collegium Medicum Jagiellonian University, Cracow, Poland

The effect of nitric oxide donor — sodium nitroprusside (NaNP) and PGE_2 on the LDL- receptor activity and LDL cellular accumulation by isolated human blood lymphocytes was investigated. Preincubation of lymphocytes with lipoprotein deficient medium (LPDS) resulted in the increase of the LDL-receptor activity and the LDL-cellular accumulation. NaNP (30—300 μM) dose-dependently prevented the increase of the LDL-receptor activity as well as the accumulation of LDL by lymphocytes. However, in "starwing" cells (cells with high LDL-receptor activity) the effect of NaNP on the receptor activity was biphasic. At concentration up to 100 μM NaNP inhibited, while at a concentration of 300 μM, it activated the LDL-receptor activity. PGE_2 (3—30 μM) inhibited LDL catabolism, however, this effect was hardly concentration-dependent.

Key words: *sodium nitroprusside, PGE_2, LDL-accumulation/degradation, LDL-receptor, lymphocytes.*

INTRODUCTION

In atherosclerosis, disturbances of cholesterol metabolism result in cholesterol and cholesterol ester accumulation in cells, especially in vascular smooth muscle cells and macrophages (1). Low density lipoprotein (LDL), the major lipoprotein which transports cholesterol in plasma, provides cholesterol to extraheptic cells through LDL-receptor binding, internalisation and lysosomal degradation (2, 3). The intracellular cholesterol level causes the down-regulation of the cell LDL-receptor binding (4) as well as the cellular synthesis of endogenous cholesterol (5, 6). Thus, the regulation of LDL catabolism plays as important role in the maintenance of the cholesterol homeostasis.

Krone et al. (7) demonstrated that PGE_1 inhibits both LDL receptor activity, the specific accumulation and degradation of LDL and the biosynthesis of cholesterol and lanosterol in lymphocytes. This activity is suggested to be mediated by c-AMP (8). Iloprost (the stable PGI_2 derivative) (9), PGE_1 and PGE_2 (10) accumulate c-AMP level in different cells including lymphocytes (7, 8). Hajjar et al. (10) described the inhibitory effect of PGI_2 on cholesterol ester synthesis in smooth muscle cells. PGI_2 and PGE_1 (11, 12) as well as PGE_2 (13) have been reported to inhibit binding of LDL to its receptor in human endothelial cells (14).

The vascular endothelial cells release another substance named "endothelium derived relaxing factor" (EDRF) (15, 16), identified as NO (17). EDRF/NO resembles the biological properties of PGI_2 such as vasodilatation and inhibition of aggregation and adhesion of platelets (11). However EDRF/NO uses c-GMP as its intracellular "second messenger" (18). The EDRF/NO activity can be substituted by nitrovasodilators (so called "NO-donors") such as SIN-1 (19) or sodium nitroprusside (NaNP). Not only PGI_2 (20) but also EDRF/NO (21) activity was found to be decreased in atherosclerosis.

On the contrary to the well recognised influence of prostaglandins and PGI_2 on LDL catabolism (22, 23), there are only a few data describing the influence of EDRF/NO or "NO-donors" on the LDL-cellular catabolism. In the present study we compared the effect of "NO-donor" (NaNP) with that of PGE_2 on LDL catabolism in lymphocytes (7, 8) which can bind and accumulate LDL only by native LDL-receptor, similarily to smooth muscle cells of vessel wall (14).

MATERIALS AND METHODS

Materials

^{125}I (5 mCi) was from NEN Boston, MA Du Pont. RPMI-1640 and Dulbecco's modified Eagle's medium (DMEM) was from Gibco (Grand Island, NY, USA). All other chemicals were from Sigma (St. Louis, MO, USA).

Cells

Venous, citrated (1 :9) blood (400 – 500 ml) was drawn from healthy volunteers. Lymphocytes were separated by the Ficoll-Paque procedure (24), washed three times with phosphate buffer solution (PBS) and suspended ($2-4 \times 10^6$ cells/dish) in RPMI 1640 medium (Gibco) containing penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% lipoprotein deficient serum (LPDS). In some experiments 10% of LPDS was replaced by 10% of foetal calf serum (FCS) (Gibco). 1 ml of the above cell suspension was plated in 35 mm dish (Nunc).

LDL isolation, and labelling

Human LDL (density 1.019 – 1.063 g/ml) were prepared by differential ultracentrifugation (25) from plasma of normolipemic volunteers. After extensive dialysis against PBS LDL was labelled with ^{125}I by the lodogen method (26). The final specific activity varied between 50 – 100 cpm/ng of LDL protein. The LDL protein was determined by Lowry method (27).

Measurement of LDL catabolism by lymphocytes

Catabolism of LDL by lymphocytes was performed by the measurement of the receptor activity and the cellular accumulation in cells with low and high LDL-receptor binding activity.

a. The influence of NaNP (0 – 300 μM) and PGE_2 (0 – 30 μM) on LDL catabolism of freshly isolated human lymphocytes.

   The lymphocytes with low LDL-receptor activity were the cells incubated during 22 hrs at 37°C in 5% CO2 atmosphere with medium containing 10% FCS (4, 5). The incubation of isolated lymphocytes with 10% LPDS during 22 hrs in these conditions resulted in the increase of the LDL-receptor number (the "up-regulation" of the LDL-receptor (4, 5)). To measure the effect of the investigated drugs on the LDL-receptor expression and LDL-accumulation in these cells, NaNP (0 – 300 μM) or PGE_2 (0 – 30 μM) were added to the incubation mixture immediately after the isolation of lymphocytes and were coincubated with cells during the whole experiment.

b. For measurement of the influence of NaNP (0 – 300 μM) or PGE_2 (0 – 30 μM) on LDL catabolism in cells with fully expressed LDL-receptor activity (4, 5) the investigated compounds were added to the cell culture after the 22 hrs preincubation of cells with 10% LPDS, and thereafter the incubation was continued for the next 22 hrs at 37°C in 5% CO2 atmosphere in the presence of investigated drugs.

In such pretreated cells (a or b) the measurement of the LDL receptor activity was performed according to Goldstein and Brown (4, 5). Briefly, 20 μg of ^{125}I-LDL was added into each dish with or without a 25-fold excess of unlabelled LDL and cells were incubated for another 6 h at 37°C. Then the medium was separated from the cells and the receptor activity of cellular fraction was measured as described below. The cell non-dependent LDL degradation was measured under identical conditions in cell – free dishes and received value was subtracted from the total counts of every dish.

For the measurement of ^{125}I-LDL – cellular accumulation the similar procedure was performed, however, the cells were incubated for 6 hours (at 37°C, 5% CO2) with 20 μg of radiolabelled LDL only (29).

To determine the labelled LDL binded to receptor or the LDL cellular accumulation, lymphocytes after incubation were washed three times with PBS and centrifuged at 800 g during 15 min at 4°C. Then, the cell pellet was dissolved in 200 μl 1N NaOH and ^{125}I-LDL radioactivity was counted in LKB γ-counter.

The results are presented as a percentage of values obtained for cells incubated in 10% LPDS medium (100%).

Data analysis

All results are presented as mean ± SD. Statistical significance was calculated by the Student's t-test and considered as $p < 0.001$ – ***; $p < 0.05$ – **; $p < 0.02$ – *.

RESULTS

Comparing with FCS medium, the 22 hour preincubation of lymphocytes in LPDS medium resulted in the increase by 40% (609±61 ng/ml cell protein/6 hours versus 1040±105 ng/mg cell protein/6 hours) the B/E receptor activity (*Fig. 1A*). NaNP (30–300 μM) dose-dependently prevented the

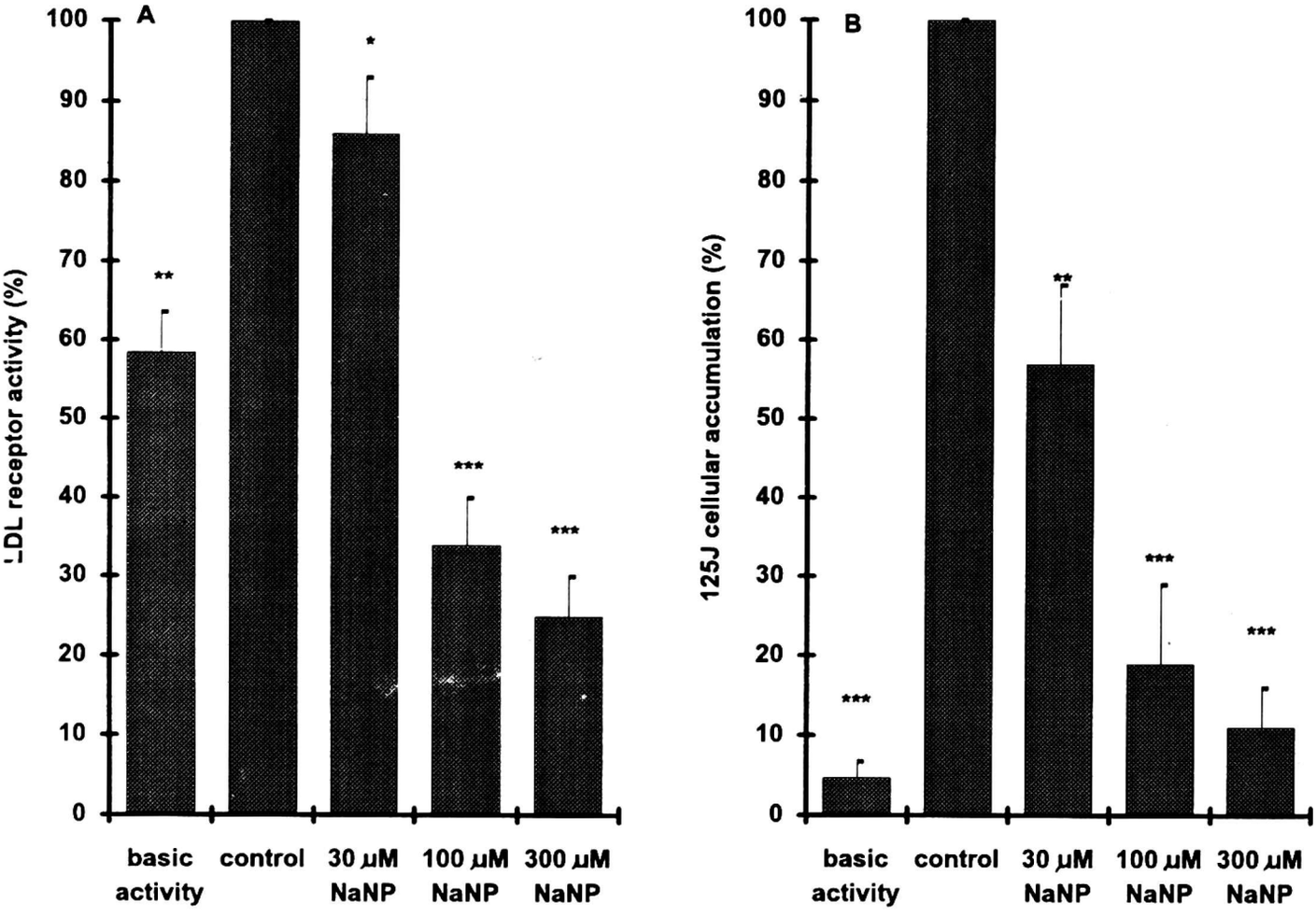

Fig. 1. Concentration dependent effect of sodium nitroprusside on the induction of LDL receptor activity (A) and ^{125}I-LDL cellular accumulation (B) induced by preincubation in lipoprotein depleted medium. The experimental conditions are described in materials and methods. Basic activity (cells incubated with 10% FCS), control cells (cells incubated with 10% LPDS). Values are the mean±SD for 2 experiments measured in triplicates for each point. Statistical significance: * $P<0.02$, ** $P<0.05$, *** $P<0.001$ vs control.

"up-regulation" of the LDL receptor activity. In higher concentrations the inhibition reached the level below the basal value (however, not significantly) (*Fig. 1A*). Similar effect was observed when the cellular accumulation of LDL (resulting from binding and accumulation of labelled lipoprotein by the cells) was investigated. The preincubation of cells with LPDS medium increased the accumulation of LDL by lymphocytes (3745±376 ng/mg cell protein/6 hours – 4.7% on *Fig. 1B*, versus 78011±7800 ng/mg cell

protein/6 hours – 100% on *Fig. 1B*). NaNP (30–300 μM) dose-dependently prevented the accumulation of LDL by cells (*Fig. 1B*). Different results were obtained when NaNP was added to the cells with the high B/E receptor activity (22 hour preincubation with 10% LPDS medium). In these experimental conditions the influence of NaNP was biphasic: at lower (30–100 μM) concentrations the inhibition of LDL receptor activity

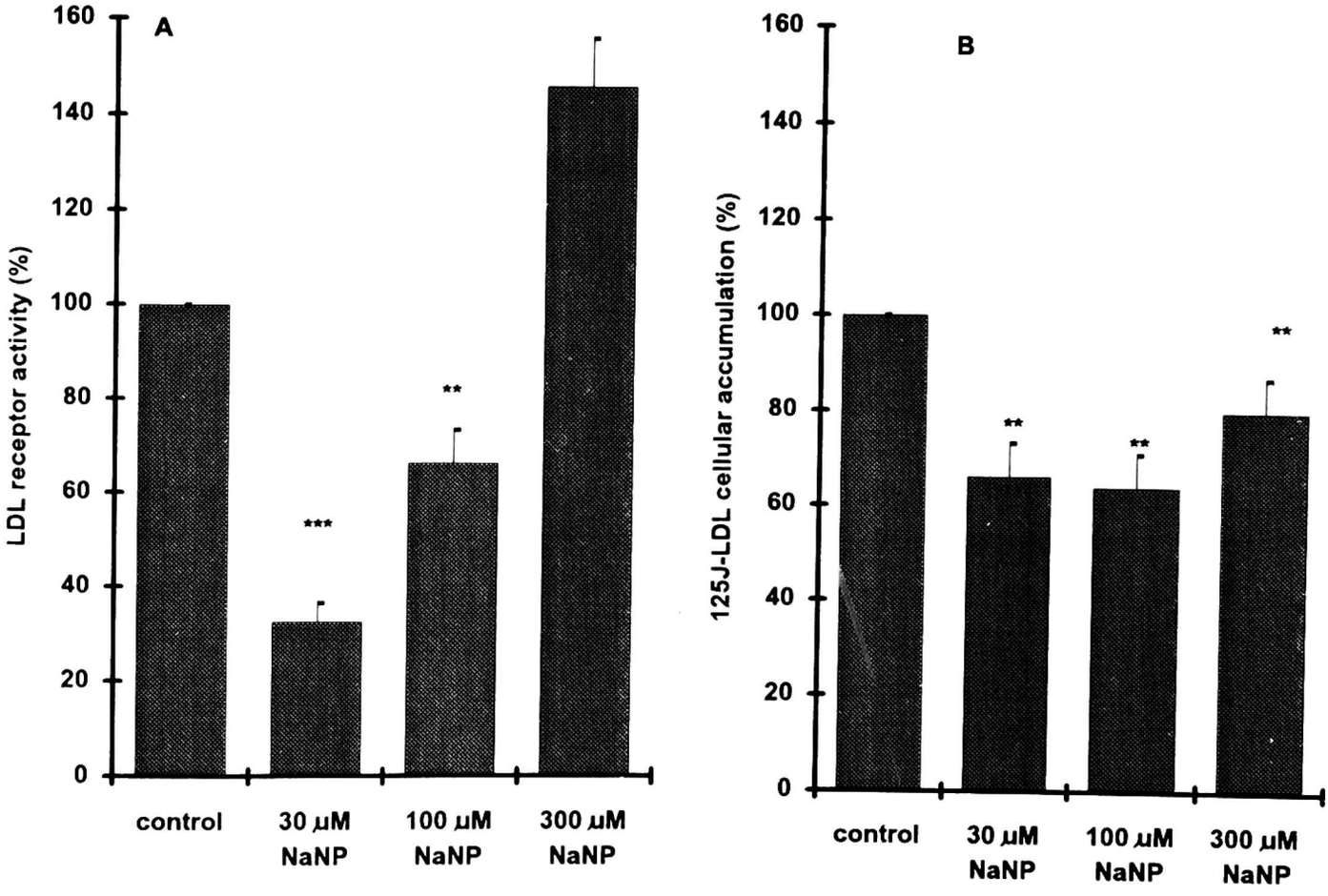

Fig. 2. Concentration dependent effect of sodium nitroprusside on LDL receptor activity (A) and ^{125}I-LDL cellular accumulation (B) by human lymphocytes with maximally expressed LDL-receptor state. For details see legend to *fig. 1.* Values are the mean ± SD for 2 experiments measured in triplicates for each point. Statistical significance: * $P<0.02$, ** $P<0.05$, *** $P<0.001$ vs control.

(*Fig. 2A*) while at 300 μM NaNP the activation of the LDL receptor activity was noticed (*Fig. 2A*). The inhibition, but not dose-dependent, of LDL accumulation by NaNP in cells with the high B/E receptor activity was observed (*Fig. 2B*).

PGE_2 (3–30 μM) inhibited totally the induction of B/E receptor activity (*Fig. 3A*) and suppressed the LDL cellular accumulation (*Fig. 3B*) almost to the basal level in starving cells. The addition of PGE_2 (3–30 μM) after maximal exposure of B/E receptor on cell membrane resulted in inhibition of both the LDL-receptor activity (300 ± 29 ng/mg cell protein/6 hours versus control

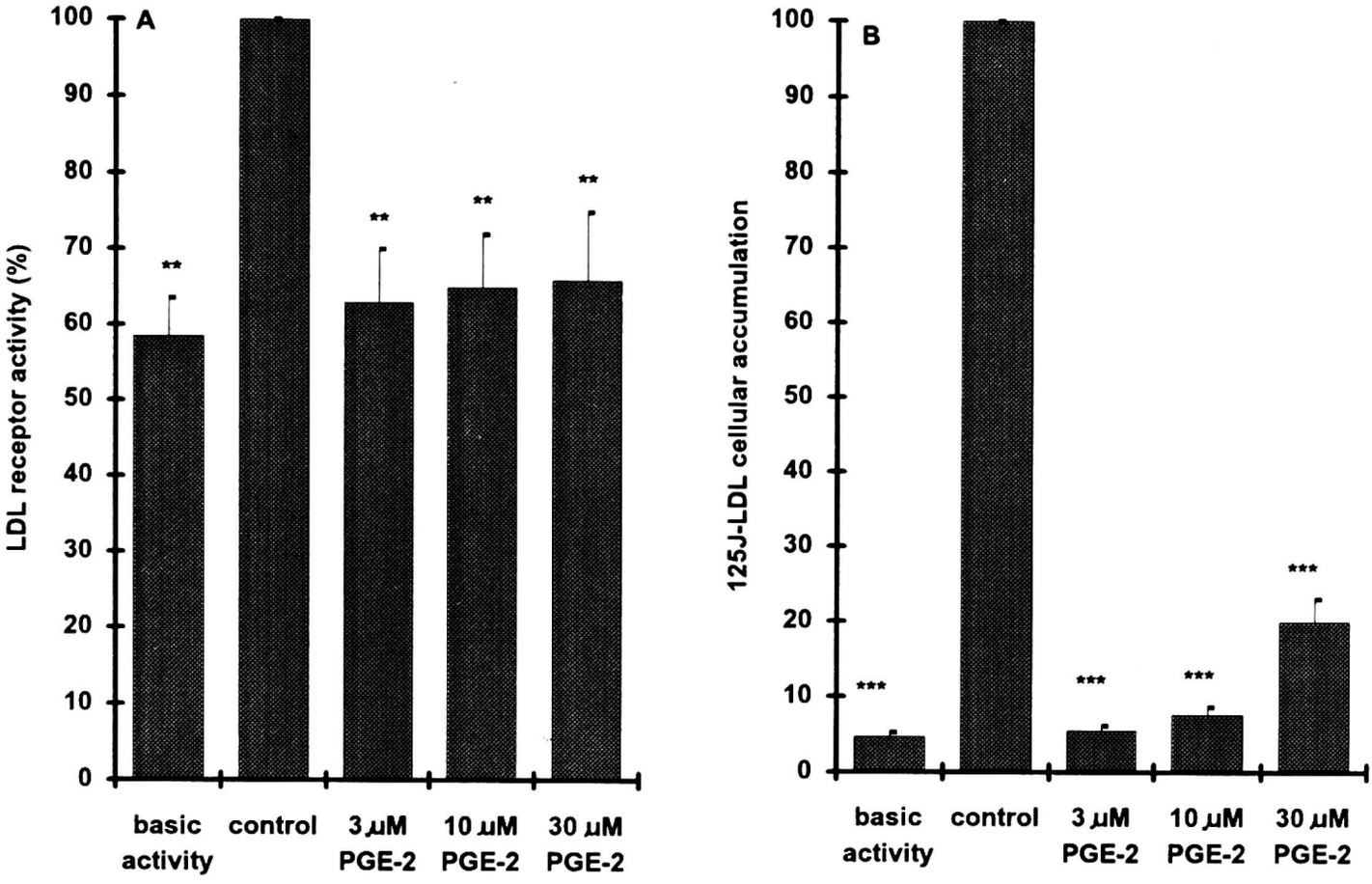

Fig. 3. Concentration dependent effect of PGE_2 on LDL receptor activity (A) and ^{125}I-LDL cellular accumulation (B) induced by preincubation in lipoprotein depleted medium. For details see legend to *fig. 1.* Values are the mean ± SD for 2 experiments measured in triplicates for each point. Statistical significance: * $P<0.02$, ** $P<0.05$, *** $P<0.001$ vs control.

640 ± 63 ng/mg cell protein/6 hours) (*Fig. 4A*) and cellular LDL accumulation (2924 ± 293 ng/mg cell protein/6 hours versus control 6279 ± 630 ng/mg cell protein/6 hours) (*Fig. 4B*) but with the low concentration-dependency. The 30 μM concentration of PGE_2 inhibited both effects by 47%.

DISCUSSION

We have confirmed the inhibitory effect of PGE_2 on LDL accumulation by human lymphocytes (23). Moreover, we have shown that sodium nitroprusside (NaNP) suppresses the LDL-receptor activity as well as LDL intracellular accumulation in these cells. Such inhibitory effect of NaNP was observed when the cellular activity of LDL receptor was low. However, at high B/E receptor activity states the biphasic action of NaNP was observed pointing that NO-donors at higher concentrations may accelerate the accumulation of cholesterol in lymphocytes (28, 29).

The biphasic effect of NO and/or "NO-donors" was reported. In the granular cells of the cerebellum and retina NO-donors exert their cytoprotective

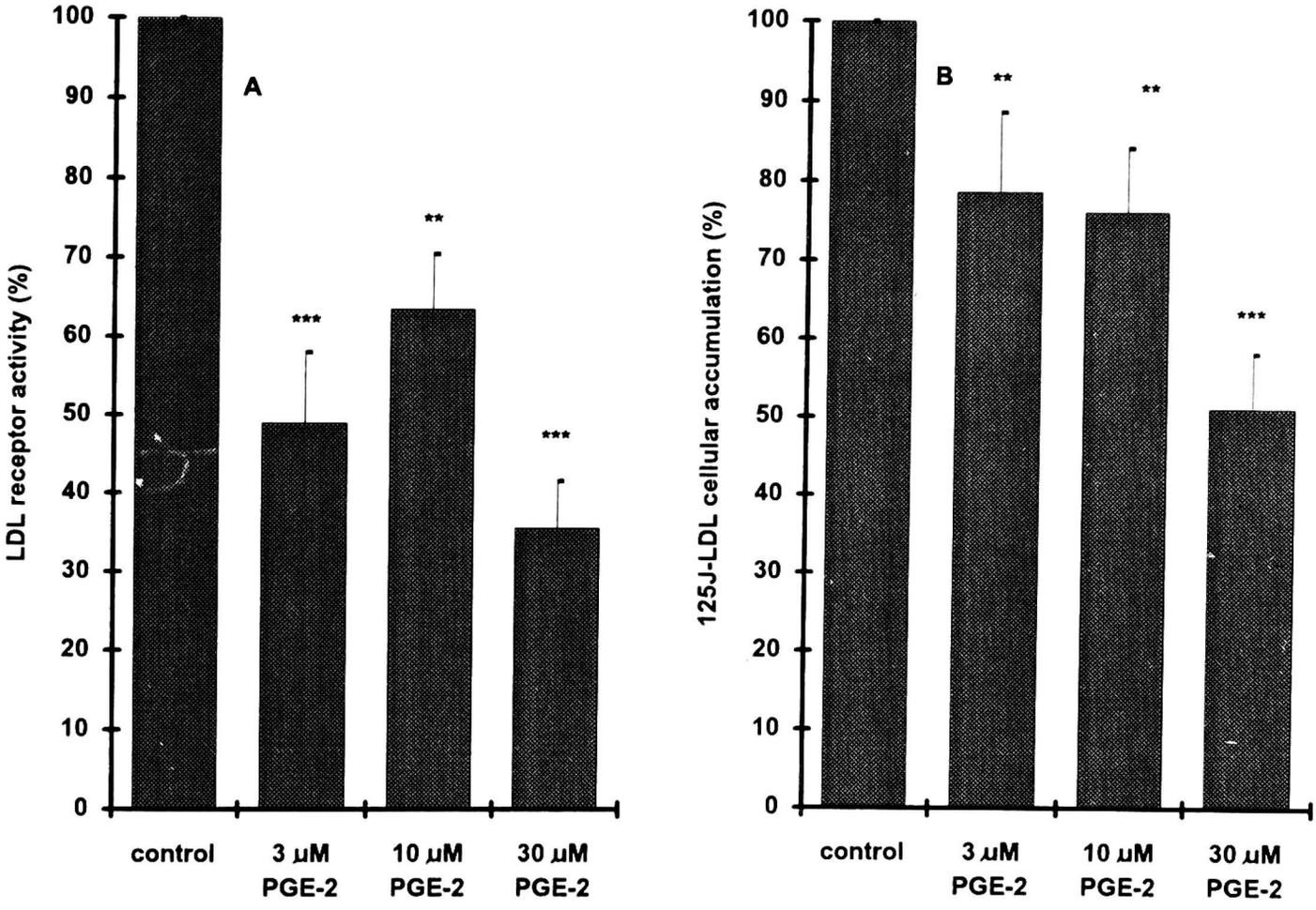

Fig. 4. Concentration dependent effect of PGE_2 on LDL receptor activity (A) and ^{125}I-LDL cellular accumulation (B) in human lymphocytes with maximally expressed LDL-receptor state. For details see legend to *fig. 1.* Values are the mean ± SD for 2 experiments measured in triplicates for each point. Statistical significance: * $P<0.02$, ** $P<0.05$, *** $P<0.001$ vs control.

effects at low concentrations up to 100 μM whereas their toxic effects appear at higher, below 300 μM, concentrations (30, 31). NO at concentrations <1 μM inhibits, and at higher concentrations it potentiates the release of noradrenaline from the perfused rabbit heart (32). Beckman et al. (33) suggested that NO-induced neurotoxicity is related to the formation of peroxynitrite ions ($ONOO_n^-$). Thus, the high amounts of NO can be toxic not only for micro-organisms (16), but also for the endothelium (33), and other cells (30, 31). In lymphocytes such toxicity may result in the impaired LDL catabolism.

The mechanism of the NO effect on LDL catabolism is not clear. The increased LDL binding at the high NO concentration may be related to the toxic deformation of either the receptor cell membrane or the LDL molecule by NO itself (binding to serine-SH groups) (34, 35) or by the $ONOO^-$.

Freshly isolated human lymphocytes exhibit a relatively low B/E receptor activity and LDL cellular accumulation (28, 29). The preincubation of lymphocytes in medium depleted of lipoproteins results in a rise in high affinity LDL-binding activity (28, 29) by the "up-regulation" of LDL-receptor activity (4, 5, our results).

The LDL receptor expression state on lymphocytes seems to determine their response to NO donors. For instance we have previously reported (36) that SIN-1 at high concentrations of 1 – 10 mM after inhibition of phosphodiesterase in lymphocytes from normolipidemic patients does increase LDL binding to their receptors. Similarly, our present study *in vitro* again shows that at the high active status of the LDL-receptor (low LDL concentrations in plasma) the higher NO concentrations may promote the accumulation of LDL by the increase of the LDL binding to its receptor. At the low B/E receptor activity (which corresponds to the high plasma LDL concentration), "NO-donors" protect cells against the lipid accumulation.

Nonetheless, we do not understand the mechanism of NO induced changes in the regulation of the LDL catabolism. The accumulation of cAMP in cells such as fibroblasts, arterial smooth muscle cells (22, 23, 37, 38), human mononuclear leucocytes (39) and lymphocytes (8), by direct application of dibutyryl-cAMP, or accumulation of endogenous c-AMP by toxin cholera, forscolin, PGE_1, PGE_2, PGI_2 analogues (carbacyclin and 6B-PGE_1) was reported to be associated with the decrease in LDL-receptor binding activity (40). The inhibition of ^{123}I-LDL uptake by PGE_1 *in vivo* in patients with atherosclerosis was also demonstrated (41). Simultaneously the decrease in intracellular cholesterol accumulation as well as the decreased synthesis of phospholipids, triglycerydes, esterified steroles, and the decrease in ^{3}H-thymidyne uptake (indicating inhibition of cell proliferation) was observed (40, 42). Thus, increase in cAMP level protects cells against invasion of lipids in a form of native LDL. EDRF/NO and "NO-donors" activate the cellular guanylate cyclase and accumulate the cellular cGMP levels (16). In platelets, PMNs or vascular smooth muscle the biological activity of NO-donors resembles that of prostacyclin and the other activators of cAMP levels. Thus, it well may be that the accumulation of cGMP in cells by the low concentrations of NO leads to the inhibition to LDL accumulation (43).

Cytokines such as Interferon-γ or TNF-α, and endotoxin which were found to stimulate biosynthesis of EDRF/NO (44, 35) cause the accumulation of lipids in plasma (45), which may, among the other mechanisms, be also related to the decreased uptake of LDL by the peripheral cells. Interferon-γ was also found to inhibit development of atherosclerosis in rabbits (46).

Thus, similarily to PGI_2, PGE_2, PGE_1, EDRF/NO and "NO-donors" may protect the cells against the accumulation of lipids. In the case of lymphocytes such protection can normalize the immunological properties of these cells, because it was found that plasma lipoproteins (VLDL and LDL) can suppress the accessory cell functions, which consequently supress the immunological function of lymphocytes (47).

It is concluded that similarily to the stimulators of adenylate cyclase such as prostacyclin and E prostaglandins the stimulators of guanylate cyclase such as

EDRF/NO and "NO-donors" may inhibit the development of atherosclerosis not only by the inhibition of platelet activity, but also by protecting cells against the excessive accumulation of lipids. However, NO at high concentrations was an opposite effect to PGE_2, PGEs and to NO at a low concentration, i.e. toxic doses of NO, may promote accumulation of intracellular lipids.

Acknowledgement: The authors wish to thank Mrs Urszula Ciałowicz and Elżbieta Siegel for excellent technical assistance. This work was supported by a grant from the Commission P2 of the Committee for Scientific Investigation of Polish Government (Nr 4 1756 9101/p 05).

REFERENCES

1. St Clari RW. Metabolism of the arterial wall and atherosclerosis. In: Atherosclerosis Reviews R Paoletti, AM Gotto Jr (eds): New York: Raven Press, 1976; pp. 61–177.
2. Goldstein JL, Brown MS. Regulation of low density lipoprotein receptors: implication for pathogenesis and therapy of hypercholesterolemia and atherosclerosis. *Circulation* 1987; 76: 504–507.
3. Rudell LL, Park JS, Johnson FL, Babiak J. Low density lipoproteins in atherosclerosis. *J Lipid Res* 1986; 27: 465–474.
4. Goldstein J, and Brown M. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu Rev Biochem* 1977; 46: 897–930.
5. Goldstein JL, Brown MS, Anderson RGW, Russell DW, Schneider WJ. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. *Annu Rev Cell Biol* 1985; 1: 1–39.
6. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986; 232: 34–37.
7. Krone W, Klass A, Nagele H, Behnke B, Greten H. Prostaglandin E_1 supresses low density lipoprotein receptor activity and cholesterol synthesis in human mononuclear leucocytes. *Prostaglandins in Clinical Research* Alan R. Liss, Inc. 1987; pp. 337–343.
8. Smith JW, Steiner AL, Newberry MJ, Parker CW. Cyclic adenosine 3′ 5′ monophosphate in human lymphocytes. Alterations after phytohemaglutinin stimulation. *J Clin Invest* 1971; 50: 433–491.
9. Hali M, Strange PG. The use of prostacyclin analogue H^3-iloprost for studying prostacyclin binding sites on human platelets and neuronal hybrid cells. *Bioscience Rep* 1984; 4: 941–948.
10. Hajjar DP, Wexler BB. Metabolic activity of cholesterol esters in aortic smooth muscle cells is altered by prostaglandins I_2 and E_2. *J Lip Res* 1983; 24: 1176–1185.
11. Gryglewski RJ, Botting RM, Vane JR. Mediators produced by the endothelial cell. *Hypertension* 1988; 12: 530–548.
12. Pardy BJ, Lewis JD, Eastcott HHG. Preliminary experience with prostaglandins E_1 and I_2 in peripheral vascular disease. *Surgery* 1980; 11: 826–928.
13. Hajjar DP, Pomerantz KB. Signal transduction in atherosclerosis: integration of cytokines and the eicosanoid network. *FASEB J* 1992; 6: 2933–2941.
14. Orehkov AN, Tortov VV, Kudryashow SA, Khasimev KHA, Smirnov VN. Primary culture of human aortic intima cells as a model for testing antiatherosclerotic drugs. Effect of cyclic AMP,

prostaglandins, calcium antagonists, antioxidants and lipid lowering agents. *Atherosclerosis* 1986; 60: 101–110.
15. Furchgott RF, Zawadzki JV. The obligatory role of entothelial cells in the relaxation of arterial smooth muscle by acetylocholine. *Nature* 1980; 288: 373–376.
16. Ignarro LJ. Endothelium-derived nitric oxide: action and properties. *FASEB J* 1989; 3: 312–336.
17. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524–526.
18. Schroder H, Leitman DC, Hayward LD, Bennett BM, Murad F. Cultured rat lung fibroblasts as a model for organic nitrate-induced cyclic GMP accumulation and activation of guanyl cyclase. *J Appl Cardiol* 1987; 2: 301–311.
19. Feelish M, Noack E. Nitric oxide (NO) formation from nitrovasodilators occurs independently of hemoglobin or non-hemic iron. *Eur J Pharmacol* 1987; 142: 465–469.
20. Dembińska-Kieć, Gryglewska T, Żmuda A, Gryglewski R. The generation of prostacyclin by arteries and by the coronary vascular bed is reduced in experimental atherosclerosis in rabbits. *Prostaglandins* 1977; 14: 1025–1034.
21. Gryglewski RJ. Nitric oxide and atherosclerosis. *Thrombotic and Haemorrhagic Disorders* 1990; 2/1: 1–9.
22. Krone W, Kaczmarczyk P, Muller-Wieland D, Greten H. The prostacyclin analog iloprost and prostaglandin E1 supress sterol synthesis in freshly isolated human mononuclear leucocytes. *Biochem Biophys Acta* 1985; 835: 154–157.
23. Krone W, Klass A, Nagele H, Behnke B, Greten H. Effect of prostaglandins on LDL receptor activity and cholesterol synthesis in freshly isolated human mononuclear leucocytes. *J Lipid Res* 1988; 29: 1663–1669.
24. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968; 97 (Suppl 21): 77–89.
25. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1958; 34: 1345–1348.
26. Fraker PJ, Speck JC. Protein and cell membrane iodination with a soluble chloramide 1, 3, 5, 6-tetrachloro 3a, 6a-diphenylglycoluril. *Biochem Biophys Res Commun* 1987; 80: 849–857.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–268.
28. Mistry P, Miller NE, Laker M, Hazzard WR, Lewis B. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol homeostasis in men. *J Clin Invest* 1981; 67: 493–502.
29. Ho YK, Brown MS, Bilheimer DW, Goldstein JL. Regulation of low density lipoprotein receptor activity in freshly isolated human lymphocytes. *J Clin Invest* 1987; 58: 1465–1474.
30. Olesen SP, Drejer J. Release of ^{3}H-D-aspartate and ^{3}H-GABA is inhibited by nitric oxide. *Journal of Endothelial Cell Research* 1993; 1: Suppl. A. 24.
31. Haberecht MF, Redburn DA, Nakane M, Schmidt HHHW. Immunocytochemical and histochemical localization of nitric oxide and soluble guanylyl cyclase in rabbit retina. *Journal of Endothelial Cell Research* 1993; 1: Suppl. A. 29.
32. Feelisch M, Bloch W, Addicks K. Control of intraaxonal catecholamine storage in cardiac sympathetic nerve fibers by endogenous nitric oxide. *Journal of Endothelial Cell Research* 1993; 1: Suppl. A. 25.
33. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 1990; 87: 1620–1624.

34. Jessup W, Mohr D, Gieseg SP, Dean RT, Stocker R. The participation of nitric oxide in cell free- and its restriction of macrophage-mediated oxidation of low-density lipoprotein. *Biochem Biophys Acta* 1992; 1180: 73–82.
35. Stamler JS, Osborne JA, Jaraki O, et al. Adverse effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 1993; 91: 308–318.
36. Dembińska-Kieć, Żmuda A, Marcinkiewicz J, Sinzinger H, Gryglewski RJ. Influence of no-donor (SIN-1) on functions of inflammatory cells. *Agents Actions* 1991; 32: 37–40.
37. Stout RW, Bierman EL. Dibutyryl-cyclic AMP inhibits LDL binding in cultured fibroblasts and arterial smooth muscle cells. *Atherosclerosis* 1983; 46: 897–930.
38. Tretov VV, Drekhov AN, Smirnov VN. Effect of cyclic-AMP on lipid accumulation and methabolism in human atherosclerotic aortic cells. *Atherosclerosis* 1986; 62: 55–64.
39. Krone W, Klass A, Nagele H, Behnke B, Greten H. Effects of prostaglandin E_1 on low density lipoprotein receptor activity and cholesterol synthesis in freshly isolated human mononuclear leucocytes. In: Prostaglandin E_1 in atherosclerosis. H. Sinzinger, W. Rogatti (eds.) Springer Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo 1986, pp. 32–37.
40. Tertar VV, Orekhov AN, Smirnov VA. Agents that increase cellular cyclic AMP inhibit proliferative activity and decrease lipid content in cells cultured from atherosclerotic human aorta. *Artery* 1986; 13: 365–372.
41. Sinzinger H, Virgolini I, Lupattelli, Molinari E, Gerakakis A, Angelberger P. Prostaglandin E_1 decreases the the low-density-lipoprotein entry into rabbit arterial wall. *Br J Pharmacol* 1991; 103: 1626–1628.
42. Hajjar DP, Weksler BB. Metabolic activity of cholesteryl esters in aortic smooth cells is altered by prostaglandins I_2 and E_2. *J Lipid Res* 1983; 24: 1176–1185.
43. Koo J, Mahoney E, Steinberg D. Neutral cholesterol esterase activity and its enhancement of c-AMP dependent protein kinase C. *J Biol Chem* 1981; 256: 12659–12661.
44. Mulligan MS, Moncada S, Ward PA. Protective effects of inhibitors of nitric oxide synthase in immune complex-induced vasculitis. *Eur J Pharmacol* 1992; 107: 1159–1162.
45. Gouni I, Oka K, Etienne J, Chan L. Endotoxin-induced hypertriglyceridemia is mediated by suppression of lipoprotein lipase at a post-transcriptional level. *J Lip Res* 1993; 34: 139–146.
46. Wilson A, Schaub R, Goldstein R, Kuo P. Suppression of aortic atherosclerosis in cholesterol-fed rabbits by purified rabbit interferon. *Arteriosclerosis* 1990; 10: 208–214.
47. Nagayasu T, Macy M, Okano R, MacCarthy BM, Harmony BA. Plasma lipoproteins can suppress accessory cell function and consequently suppress lymphocyte activation. *Exp Cell Res* 1986; 163: 103–116.

Received: January 7, 1994
Accepted: March 17, 1994

Author's address: A. Dembińska-Kieć, Department of Clinical Biochemistry, Collegium Medicum Jagiellonian University, 15A Kopernika Str., 31-501 Cracow, Poland