EFFECTS OF PHTHALAZINOL (EG 626) AND PYRIDINOLCARBAMATE (ANGININ) ON LIPOLYTIC ENZYME ACTIVITIES IN RAT ADIPOSE TISSUE -IN VIVO AND IN VITRO-

Takako TOMITA, Ikumi YONEKURA, Yasufumi SHIRASAKI
and Eiichi HAYASHI

Laboratory of Pharmacology, Shizuoka College of Pharmaceutical Sciences,
2-2-1 Oshika, Shizuoka 422, Japan

Fujio NUMANO

Department of Internal Medicine (3), Tokyo Medical and Dental University,
1-chome, Yushima, Bunkyo-ku, Tokyo 113, Japan

Accepted October 8, 1979

Abstract—Effects of Phthalazinol (EG 626), a cyclic AMP phosphodiesterase inhibitor, a thromboxane A_2 antagonist and an antiatherosclerotic agent was examined regarding lipolytic enzyme activities in rat epididymal adipose tissue. The effect of Pyridinolcarbamate (Anginin) was concomitantly examined. There was a significant decrease in serum triacylglycerol levels in rats given EG 626 (100–500 mg/kg), p.o. for 1–3 weeks. In adipose tissue from EG 626 treated rats, the basal and adrenalin induced lipolysis, and cholesterylester hydrolase activity were markedly enhanced, while the phosphodiesterase activity was decreased. Anginin treatment had no effect either on the serum lipid levels or the cholesterylester hydrolase activity. An elevation in cholesterylester hydrolase activity and lipolysis by EG 626 was observed both *in vivo* and *in vitro*. Incubation of the adipose tissue with 0.229 mM of EG 626 or 0.603 mM of theophylline induced a lipolysis equivalent to that seen with 2.7×10^{-2} mM of adrenalin. These results indicate that EG 626 exerted marked effects on lipolysis and cholesterylester hydrolase activity, probably through inhibition of phosphodiesterase. Possible contributions of the enhanced cholesterylester hydrolase activities to the antiatherogenic effect were discussed.

Phthalazinol (EG 626) is a phthalazin derivative with the chemical structure shown in Fig. 1. Pharmacological studies have shown that EG 626 has an antagonistic effect on

FIG. 1. Chemical structures of Phthalazinol (EG 626) and Pyridinolcarbamate (Anginin)

thromboxane A_2 (1, 2) and a potent inhibitory effect on cyclic-AMP phosphodiesterase activity (3, 4). EG 626 prevents the contraction of rabbit aortic strips induced by thromboxane A_2 (1). Shimamoto (2) observed that myocardial infarction induced in rabbits by an intra-coronary injection of thromboxane A_2 could be prevented by pretreating the animals with EG 626. The inhibitory effect on cyclic-AMP phosphodiesterase has been demonstrated in various organs including the aorta and platelets (3). EG 626 has further been shown to possess an inhibitory effect on platelet aggregation (5), a preventive effect on arterial cholesterol accumulation (6) and on the decrease of cyclic-AMP in the atheromatous lesion in the aorta of the cholesterol-fed rabbit (7).

Rat adipose tissue contains multiple acyl hydrolase activities—lipoprotein lipase (LPL), hormone-sensitive triacylglycerol lipase, diacylglycerol hydrolase, monoacylglycerol hydrolase (8–11), cholesterylester hydrolase (12, 13). Cholesterylester hydrolase activity was first demonstrated in human adipose tissue by Arnaud and Boyer (12). Pittman et al. found that the enzyme from rat adipose tissue was activated to about the same degree as the triacylglycerol lipase from adipose tissue by cyclic-AMP dependent protein kinase (13). Cholesterylester hydrolase from rat adrenal is also phosphorylated to an active form by cyclic-AMP dependent protein kinase (14). Therefore, it was of interest to examine the effect of EG 626, a potent phosphodiesterase inhibitor, on the two enzymes: hormone sensitive triacylglycerol lipase and cholesterylester hydrolase in adipose tissue.

In attempts to elucidate the mechanism of the antiatherogenic effect of EG 626 in lipid metabolism, we examined *in vivo* and *in vitro* effects of EG 626 on the lipolysis and cholesterylester hydrolase activity in adipose tissue which contained triacylglycerols and cholesterol in dynamic equilibrium with plasma lipids. Effects of Pyridinolcarbamate (Fig. 1) (15) were simultaneously examined.

MATERIALS AND METHODS

Animal treatment: Male Wistar rats weighing about 150 g were maintained on the usual laboratory chow diet and were given orally EG 626 (100–500 mg/kg) or Anginin (200 mg/kg) suspended in 1% carboxymethyl cellulose during the indicated term. Control rats were given only the vehicle. The animals were fasted for 12 hr and given the last dose of drugs 5 hr before sacrifice.

Analysis of serum lipids: Serum cholesterol and triacylglycerols were determined according to the method of Zak and Dickenman (16), and Block and Jarret (17), respectively.

Assay of adipose tissue cholesterylester hydrolase activity (E.C. 3.1.1.13): The epididymal adipose tissue (1 g) was homogenized by Polytron (PT 10, Kinematica, GMBH, Lausanne, Switzerland) in 10 vol. of 0.03 M phosphate buffer (pH 7.4) and centrifuged for 10 min at 1000 g. The clear supernatant was utilized for assay of cholesterylester hydrolase. Cholesterylester hydrolase activity was measured in terms of release of [$1-^{14}C$]-oleic acid from cholesteryl-[$1-^{14}C$]-oleate (54 mCi/nmol, NEN, Boston, Mo., U.S.A.). The substrate solution was prepared according to the method of Brecher *et al.* (18) by sonicating labelled compounds with egg yolk lecithin in 0.01 M Tris (pH 7.4)/0.1 M NaCl/0.01% sodium azide.

The reaction mixture for cholesterylester hydrolase assay contained 50–100 μ l the supernatant, 20 μ l the substrate solution (cholesteryl-[1- 14 C]-oleate, about 5×10^4 dpm/3.5 nmol) and 100 μ l 0.1 M phosphate buffer (pH 6.5) (total 0.2 ml). The reaction was carried out for 1.5 hr at 37°C, and stopped by the addition of 0.6 ml 0.3 M NaOH. An extraction solvent (CHCl_3 : MeOH : Benzene, 0.5 : 1.2 : 1) containing 0.1 mM oleic acid (3 ml) was then added and the mixture shaken vigorously. The radioactivity (released [1- 14 C]-oleic acid) in the aqueous layer was counted using a liquid scintillation counter (Aloka LSC, 602, Nihon Musen, Tokyo).

Measurement of adrenalin induced lipolysis: The method used was as described elsewhere (19). In short, a 100 mg of epididymal adipose tissue was preincubated for 15 min at 37°C in 3 ml Krebs-Ringer Henseleit bicarbonate buffer (pH 7.4)/0.54 mg glucose/ml. Each piece was transferred to a tube with 1 ml of the same buffer containing various amounts of adrenalin (Merck, Darmstadt, Germany) and incubated for 2 hr at 37°C under a gas phase of 95% O_2 –5% CO_2 . The reaction was stopped by quickly chilling the tube in ice and the tissue was removed. Glycerol released in the medium was assayed using the enzyme spectrophotometric technique (20).

Assay of adipose tissue 3',5' cyclic-AMP phosphodiesterase (E.C. 3.1.4.17) activity (21): About 200 mg of the epididymal adipose tissue excised from rats after a 12 hr fast was homogenized in 5 vol. of 0.03 M phosphate buffer (pH 7.5) and the homogenate was centrifuged for 20 min at 1000 g. The supernatant was assayed for phosphodiesterase activity. The reaction mixture for phosphodiesterase assay contained 10 μ l enzyme solution and 30 μ l substrate solution containing 0.3 μ Ci [8- ^3H]-cyclic AMP (30 mCi/ μ mol, The Radio-Chemical Center, Amsterdam), 0.09 nmol cyclic-AMP (Kohjin Co. Tokyo), 3 μ g snake venom (Sigma, St. Louis, Mo.) and 0.2 μ mol MgCl_2 (Wako Chemicals, Tokyo) in 50 mM Tris-HCl buffer (pH 7.4). The reaction was carried out for 10 min at 37°C and stopped by the addition of 10 μ l 2N HCl in the cold. The mixture (5 μ l) was applied on a TLC plate (Polygram cell DEAE 300, 20 $\times$ 20 cm, Macherey-Nagel Co., Düren, Germany) and the plate was developed with 1 M ammonium acetate: 95% EtOH (30 : 75). Spots were visualized under a ultra violet lamp (2536Å) and identified with standards; cyclic-AMP, adenosine (Kohin, Co., Tokyo) and 5'-AMP (Boehringer Mannheim Co., Mannheim, Germany). Each spot was scraped into a counting vial and the radioactivity was counted.

RESULTS

Physical parameters of the experimental animals: Administration of EG 626 (100–500 mg/kg) for less than 3 weeks did not affect growth, while 3 weeks of Anginin treatment (200 mg/kg) slightly enhanced the growth of these rats. There was no liver enlargement such as was seen in the case of Clofibrate (22, 23). When EG 626 and Anginin were administered less than 2 weeks, the weight of the epididymal adipose tissue tended to decrease.

Serum lipid levels: Table 1 shows changes in the serum cholesterol and triacylglycerol levels. There was a significant and consistent decrease in the triacylglycerol level after administration of EG 626, while the serum cholesterol level was decreased only with a 3

TABLE 1. Effect of EG 626 and Anginin on serum lipid levels

Treated				No. of Rats	Cholesterol	Triacylglycerols
	Term (days)	Drug	Dose (mg/kg)			
mg % (mean±SE)						
Exp. 1	6	None		8	50±2	66±3
		EG 626	100	8	49±2	56±2* (-15%)
			500	8	55±2	41±2** (-38%)
	12		100	7	51±3	44±4** (-33%)
Exp. 2	14	None		13	57±1	78±2
		EG 626	200	13	59±3	62±1** (-21%)
		Anginin	200	6	58±1	80±4
Exp. 3	21	None		8	51±1	60±2
		EG 626	200	8	44±2** (-14%)	53±2* (-12%)
		Anginin	200	7	50±2	60±1

* Significance $p<0.05$, ** Significance $p<0.01$

week treatment of EG 626 (200 mg/kg). Anginin had no effect on either level. Serum non-esterified fatty acid was significantly lower in the 2 week EG 626 treated group (200 mg/kg) than in the control group (0.72 ± 0.047 [7]* vs. 1.00 ± 0.105 [7] $\mu\text{mol/ml}$, $P<0.05$).

Adrenalin-induced lipolysis in the epididymal adipose tissue: Figure 2 shows the effect of EG 626 and Anginin on adrenalin-induced lipolysis of the epididymal adipose tissue. EG 626 treatment significantly enhanced the basal lipolytic activity (the activity in the absence of adrenalin), while Anginin had no effect. Incubation with a low concentration of adrenalin (2.7×10^{-4} mM=0.05 $\mu\text{g/ml}$) did not alter the lipolytic activity in the adipose tissue of control rats, whereas it greatly enhanced lipolysis in tissues from EG 626 or Anginin treated rats. At a high concentration of adrenalin (2.7×10^{-2} mM=5 $\mu\text{g/ml}$) lipolysis was fully activated, while EG 626 treatment apparently had no further effects on the maximum

FIG. 2. Effect of EG 626 and Anginin on the lipolytic response to adrenalin in epididymal adipose tissue *in vivo*. After a 12 hr fast, the epididymal adipose tissue was excised and approximately 100 mg of the tissue was incubated for 2 hr at 37°C in 1 ml of Krebs-Ringer Henseleit bicarbonate buffer (pH 7.4) in the presence or absence (basal activity) of adrenalin. Glycerol released in the medium was assayed using the spectrophotometric technique (18).

lipolysis. Anginin treatment slightly enhanced this activity.

Cholesterylester hydrolase activity of the epididymal adipose tissue: Figure 3 shows the change in cholesterylester hydrolase activity of the epididymal adipose tissue due to EG 626 treatment. The treatment markedly enhanced the cholesterylester hydrolase activity, particularly in the short term experiment, while Anginin treatment had no effect.

Phosphodiesterase activity of the epididymal adipose tissue: As the increase in lipolysis and cholesterylester hydrolase activity in the adipose tissue from EG 626 treated rats suggested elevation of the cyclic AMP level, phosphodiesterase activity in the tissue was measured in tissues from the 3 week EG 626 treated rats (200 mg/kg) and the control rats. Phosphodiesterase activity in the treated rats (0.861 ± 0.0411 [7] nmol adenosine/mg protein/10 min) was significantly lower than that in control rats (1.061 ± 0.0092 [6] nmol adenosine/mg protein/10 min).

Effects of EG 626 on cholesterylester hydrolase activity and lipolysis in vitro: As the administration of EG 626 exerted marked effects on lipolysis and cholesterylester hydrolase activity in the adipose tissue, the *in vitro* effect was examined. When the fat pad was incubated with EG 626 (1 mM) for 20 min at 37°C, the cholesterylester hydrolase activity was

FIG. 3. Effect of EG 626 on cholesterylester hydrolase activity of epididymal adipose tissue *in vivo*. After a 12 hr fast, the epididymal adipose tissue and 1 g of the tissue was homogenized with 10 vol. of 0.03 M phosphate buffer (pH 7.4). The homogenate was centrifuged for 10 min at 1000 g. The clear supernatant was used for the assay of cholesterylester hydrolase.

TABLE 2. Effects of EG 626 on cholesteryl ester hydrolase activity of the epididymal adipose tissue *in vitro*

Incubated with			Cholesterylester hydrolase activity (nmol fatty acids/mg protein/1.5hr)
			Mean $\pm$ SE [No. of determinations]
None			6.41 $\pm$ 0.248 [15]
EG 626	1 mM		8.04* $\pm$ 0.329 [10]
Theophylline	1 mM		7.92* $\pm$ 0.393 [15]
Adrenalin	2.7×10^{-2} mM		7.71* $\pm$ 0.329 [15]

Small pieces of the epididymal adipose tissue were preincubated for 30 min at 37°C with EG 626 or the other drugs in 0.03 M phosphate buffer (pH 7.4) and the tissue were rinsed with the same buffer. The supernatant of the tissue homogenate was assayed for cholesterylester hydrolase activity.

* Significant $p < 0.01$

FIG. 4. Effect on lipolysis in epididymal adipose tissue *in vitro*. Approximately 100 mg pieces of the epididymal adipose tissue were incubated for 2 hr at 37°C in 1 ml of Krebs-Ringer Henseleit bicarbonate buffer (pH 7.4) containing EG 626 or theophylline under a gas phase of 95% O₂-5% CO₂. Glycerol was measured using the spectrophotometric method (18). Each point represents the mean of 3 determinations.

elevated by 25% (Table 2) compared to the activity seen when incubation was carried out without addition of the drug. The activation by EG 626 was comparable to that seen with theophylline (1 mM) or adrenalin (2.7×10^{-2} mM). Figure 4 shows the *in vitro* effect of EG 626 on lipolysis of adipose tissue. An incubation of the fat pad with EG 626 (0.05–1.0 mM), theophylline (1.0–1.0 mM) or adrenalin (2.7×10^{-4} and 2.7×10^{-2} mM) gradually enhanced lipolysis with the increasing concentrations. The ratio of lipolytic activity in the presence of the drugs to the basal activity in their absence was linear to the log dose of the drugs. Equal lipolytic response to adrenalin (2.7×10^{-2} mM) was produced with 0.229 mM EG 626 and 0.603 mM theophylline.

DISCUSSION

Adipose tissue is the major storage organ not only for triacylglycerols but also for cholesterol (24). The pools of triacylglycerols and cholesterol in adipose tissue are in dynamic equilibrium with plasma lipids through functions of lipolytic enzymes in the tissue. Adrenalin-induced lipolysis is reported to be mediated by cyclic AMP through activation of adrenergic β -receptors (25). In adipose tissue from our rats treated with EG 626, basal lipolysis was significantly enhanced compared with that of control rats, and in the treated rats only, lipolysis was greatly enhanced by a lower concentration of adrenalin. With a higher concentration of adrenalin, lipolysis was stimulated to the same extent in tissues from both the treated and control animals. Since phosphodiesterase activity was lower in adipose tissue following the EG 626 treatment, the enhancement in lipolysis, both basal and adrenalin-induced, and in the activity of cholesterylester hydrolase was probably mediated by the inhibitory effect of cyclic AMP phosphodiesterase. Cyclic AMP produced with incubation of a low concentration of adrenalin would therefore be less likely to decompose in the

epididymal adipose tissue from the treated animals and could induce the lipolytic response. With a higher concentration of adrenalin, an excessive amount of cyclic AMP would be produced. Thus, with such a slight difference in the catabolic rate of the nucleotides, the rate of lipolysis was not influenced. Anginin treatment significantly enhanced adrenalin-induced lipolysis at the high and low concentrations but there was no effect on the basal lipolysis. As this compound apparently has no effect on phosphodiesterase, the mechanism of the enhancement by Anginin probably differs from that by EG 626.

Cholesterylester hydrolase activity was enhanced by EG 626 treatment to almost the same extent as the basal lipolysis except for the two week treatment, and the activation of the enzyme was observed on incubation of adipose tissue not only with EG 626 but also with other cyclic AMP modulators; adrenalin and theophylline. Accordingly, our observations suggest that the activation of cholesterylester hydrolase by EG 626 is the result of cyclic AMP dependent phosphorylation of the enzyme protein.

Nikkila *et al.* observed that there was a highly significant positive correlation between the plasma HDL cholesterol level and LPL activity in adipose tissue, and they proposed that the rate of catabolism of triacylglycerol rich lipoprotein might be one of the factors to determine the concentration of HDL in plasma (26). The physiological functions of adipose tissue cholesterylester hydrolase are obscure. It has been shown that adipose tissue can take up cholesterol from plasma lipids both *in vivo* and *in vitro* (27-29), and that the cholesterylester of lipoproteins is hydrolyzed during uptake by adipocytes (18). Thus, the enhancement of cholesterylester hydrolase activity in adipose tissue by treatment with EG 626 may produce a change in the distribution of cholesterol in HDL and LDL. In fact, though the mechanism is unclear, a long term administration of EG 626 (one year, 50 mg/kg, p.o.) resulted in a significant elevation in the ratio of HDL- to LDL-cholesterol in the rat. Thus, antiatherosclerotic effects of EG 626 would be exerted through alteration in plasma lipids as well as through alteration in arterial lipolytic enzyme activities. Effects of EG 626 on rat arterial enzyme activities were reported elsewhere (30).

Acknowledgements: We thank A. Iwamoto and A. Suzuki for technical assistance.

REFERENCES

- 1) SHIMAMOTO, T., TAKASHIMA, Y., KOBAYASHI, M., MORIYA, K. AND TAKAHASHI, T.: A thromboxane A₂-antagonistic effect of pyridinolcarbamate and phthalazinol. *Proc. Japan Acad.* **52**, 591-594 (1976)
- 2) SHIMAMOTO, T.: Stroke and heart attack induced experimentally by thromboxane A₂ in rabbits and effect of EG 626, a thromboxane A₂ antagonist. *J. Japan Atheroscler.* **5**, 107-116 (1977)
- 3) ADACHI, K. AND NUMANO, F.: Phosphodiesterase inhibitors: Their comparative effectiveness *in vitro* in various organs. *Japan J. Pharmacol.* **27**, 97-103 (1977)
- 4) ASANO, T., OCHIAI, Y. AND HIDAKA, H.: Selective inhibition of separated forms of human platelet cyclic nucleotide phosphodiesterase by platelet aggregation inhibitors. *Mol. Pharmacol.* **13**, 400-406 (1977)
- 5) SHIMAMOTO, T., KOBAYASHI, M., MORIYA, K. AND MATSUZAKI, M.: Coexistence of anti-thrombotic and antihemorrhagic effect in phthalazinol, a competitive cyclic-AMP phosphodiesterase inhibitor. *J. Japan Atheroscler. Soc.* **3**, 437-446 (1976)

- 6) KOBAYASHI, M., MORIYA, K., TAKAHASHI, T., SAKAMOTO, M., TAKASHIMA, Y., NUMANO, F. AND SHIMAMOTO, T.: Preventive effect of phthalazinol (EG 626) in experimental atherosclerosis. *J. Japan Atheroscler.* **5**, 233-240 (1977)
- 7) NUMANO, F., MAEZAWA, H., SHIMAMOTO, T. AND ADACHI, K.: Changes of cyclic-AMP and cyclic-AMP phosphodiesterase in the progression and regression of experimental atherosclerosis. *Ann. N.Y. Acad. Sci.* **275**, 311-320 (1976)
- 8) KORN, E.D. AND QUIGLEY, T.W. JR.: Studies on lipoprotein lipase of rat heart and adipose tissue. *Biochim. Biophys. Acta*, **18**, 143-145 (1955)
- 9) VAUGHAN, M., BERGER, J.E. AND STEINBERG, D.: Hormone-sensitive lipase and mono-glyceride lipase activities in adipose tissue. *J. biol. Chem.* **239**, 401-409 (1964)
- 10) RIZACK, M.A.: An epinephrine-sensitive lipolytic activity in adipose tissue. *J. biol. Chem.* **236**, 657-662 (1961)
- 11) HUTTUNEN, J.K. AND STEINBERG, D.: Activation and phosphorylation of purified adipose tissue hormone-sensitive lipase by cyclic AMP-dependent protein kinase. *Biochim. Biophys. Acta*, **239**, 411-427 (1971)
- 12) ARNAUD, J. AND BOYER, J.: Identification of an acylcholesterol lipase activity in human adipose tissue. *Biochim. Biophys. Acta*, **337**, 165-168 (1974)
- 13) PITTMAN, R.C., KHOO, J.C. AND STEINBERG, D.: Cholesterol esterase in rat adipose tissue and its activation by cyclic-AMP dependent protein kinase. *J. biol. Chem.* **250**, 4505-4511 (1975)
- 14) PITTMAN, R.C. AND STEINBERG, D.: Activatable cholesterol esterase and triacylglycerol lipase activities of rat adrenal and their relationship. *Biochim. Biophys. Acta*, **487**, 431-444 (1977)
- 15) SHIMAMOTO, T.: Prevention and enhancement of regression of human atherosclerosis by modifying local factors. *Atherosclerosis Reviews*, Edited by Paoletti, R and Gotto, A. M. Jr., Vol. **2**, p. 233-251, Raven Press, New York (1977)
- 16) ZAK, B. AND DICKENMAN, R.C.: Rapid estimation of free and total cholesterol. *Am. J. clin. Pathol.* **24**, 1307-1315 (1954)
- 17) BLOCK, W.D. AND JARRET, K.J.: An automated technique for the quantitative determination of serum total triglycerides. *Am. J. med. Technol.* **35**, 93-102 (1969)
- 18) BRECHER, P., CHOBANIAN, J., SMALL, D.M. AND CHOBANIAN, A.V.: The use of phospholipid vesicles for *in vitro* studies on cholesterol ester hydrolysis. *J. Lipid Res.* **17**, 239-247 (1976)
- 19) HAYASHI, E., MAEDA, T., HASEGAWA, R. AND TOMITA, T.: The effect of myo-inositol deficiency on lipid metabolism in rats. III. The mechanism of an enhancement in lipolysis due to myo-inositol deficiency in rats. *Biochim. Biophys. Acta*, **531**, 197-205 (1978)
- 20) EGGSTEIN, M. AND KUHLMANN, E.: *Triglycerides and Glycerol*, Determination after alkaline hydrolysis, Edited by BERGMAYER, H. U. vol. **4**, p. 1823-1831, Academic Press, New York and London (1974)
- 21) NUMANO, F., TAKENO, K., TAKANO, T., WATANABE, Y., NAKAJIMA, M., ARITA, K., MAEZAWA, H. AND ADACHI, K.: Microassay of cyclic nucleotides in vessel wall. II. Cyclic AMP phosphodiesterase activity. *Microvascular Research*, **15**, 229-237 (1978)
- 22) TOMITA, T., TAKESHITA, R., NAGAKURA, H. AND HAYASHI, E.: Pharmacological study of bis-[2-(p-chlorophenoxy)-isobutyric acid]-hydroxy aluminium on the lipid metabolism in normal rats. *Japan. J. Pharmacol.* **23**, 281-287 (1973)
- 23) TOMITA, T., YAMAMURA, Y. AND HAYASHI, E.: Pharmacological study of bis-[2-(p-chlorophenoxy)-isobutyric acid]-hydroxy aluminium on the lipid metabolism in hypercholesterolemic rats. *Japan J. Pharmacol.* **23**, 289-298 (1973)
- 24) FARKAS, J., ANGEL, A. AND AVIGAN, M.I.: Compartmentation of lipid in adipose tissue cells. II. Cholesterol accumulation and distribution in adipose tissue components. *J. Lipid Res.* **14**, 344-356 (1973)
- 25) FAIN, J.N.: Dihydroergotamine, propranolol, and the beta adrenergic receptors of fat cells. *Fed. Proc.* **29**, 1402-1407 (1970)
- 26) NIKKILÄ, E.A., TASKINEN, M.R. AND KEKKI, M.: Relation of plasma HDL cholesterol to LPL activity in adipose tissue and skeletal muscle of man. *Atherosclerosis* **29**, 497-501 (1978)

- 27) QUARFORDT, S.H. AND GOODMAN, D.S.: Metabolism of doubly-labeled chylomicron cholesterol esters in the rat. *J. Lipid Res.* **8**, 264-273 (1967)
- 28) BROT, N., LOSSOW, W.J. AND CHAIKOFF, I.L.: In vitro uptake and hydrolysis, by rat tissues, of cholesterol esters of a very low density chyle lipoprotein fraction. *J. Lipid Res.* **5**, 63-67 (1964)
- 29) NESTLE, P.J., WHYTE, H.M. AND GOODMAN, D.S.: Distribution and turnover of cholesterol in humans. *J. clin. Invest.* **48**, 982-991 (1969)
- 30) TOMITA, T., YONEKURA, I., SHIRASAKI, Y. AND HAYASHI, E.: Effects of Phthalazinol (EG 626) on arterial lipolytic enzyme activities in the rat. *Arterial Wall.* (in press)