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Effects of estradiol on very low density lipoprotein receptor mRNA levels in rabbit heart

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Abstract

The VLDL receptor, a newly identified lipoprotein receptor, has a domain structure homologous with the LDL receptor but has one additional cysteine rich repeat in the ligand binding domain. To study the regulation of the VLDL receptor in vivo, we administered 17 α-ethinyl estradiol to rabbits and examined its effects on VLDL receptor mRNA levels in the heart, one of the organs with highly expressed VLDL receptors, by RNA blotting. The ventricular level of VLDL receptor mRNA increased dramatically after estradiol administration. We could not detect VLDL receptor mRNA in the liver even after estradiol administration. We have confirmed the enhanced expression of liver LDL receptor mRNA by estradiol, however, only weak expression of the LDL receptor was detected in the ventricle of the rabbit heart. These results suggest that estradiol exerts its effect on the VLDL receptor gene expression in the heart.

Key words: Lipoprotein; Lipoprotein receptor; Very low density lipoprotein; Estrogen; LDL receptor; VLDL receptor

1. Introduction

Very low density lipoprotein (VLDL) receptor cDNA was cloned by hybridization with a rabbit low density lipoprotein (LDL) receptor cDNA probe from LDL receptor subtracted rabbit heart cDNA library [1]. The deduced amino acid sequence revealed the existence of a new lipoprotein receptor with close similarities to the LDL receptor [2] both in domain structure and exon–intron boundary organization, suggesting a common ancestral origin [3]. The striking difference was an additional cysteine-rich repeat in the ligand binding domain in the VLDL receptor. This rabbit VLDL receptor cDNA was introduced into LDL receptor-deficient cells [1]. The transfected cells bound fluorescent VLDL and β-VLDL but not LDL. Furthermore, in contrast to the LDL receptor, the VLDL receptor mRNA is expressed in the heart, muscle and adipose tissue, which are active in fatty acid metabolism. So far the regulation of VLDL receptor expression has not been studied. In order to elucidate the role of this receptor in lipid metabolism in vivo, we administered pharmacological doses of ethinyl estradiol into rabbits and analyzed the VLDL receptor expression in their hearts and livers in comparison with LDL receptor expression.

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Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; β-VLDL, β-migrating very low density lipoprotein; WHHL, Watanabe heritable hyperlipidemic; LPL, lipoprotein lipase.

2. Materials and methods

2.1. Materials

17 α-Ethinyl estradiol (catalog no.E4876) and propylene glycol were obtained from Sigma Chemical Co. (St Louis, USA). [α-^{32}P]dCTP (> 3000 Ci/m mol) was obtained from Amersham Corp. Other chemicals were all of reagent grade.

2.2. Animals and estradiol administration

Male Japanese white rabbits (2.0 kg) were exposed to 12 h of light (6.00 h to 18.00 h) and 12 h of darkness (18.00 h to 6.00 h) daily for 1 week prior to use. 17 α-Ethinyl estradiol was dissolved in propylene glycol (10 mg/ml) and administered subcutaneously around 10.00 h each day in a final volume of 1 ml with 2.5 or 5.0 mg/kg body weight.

2.3. Isolation of RNA

Tissues were dissected from rabbits anesthetized with pentobarbital in the morning of the day, quickly frozen in liquid nitrogen, and stored at −70°C. Total RNA was isolated by the guanidine thiocyanate method of Chirgwin et al. [4]. The yield of RNA ranged within 240–2480 μg/g tissue, depending on the kind of tissue. Poly (A)-containing RNA was enriched by using oligo(dT) latex (Roche). The yield of poly (A)-rich RNA was around 3.5% of total RNA.

2.4. RNA blot hybridization analysis

The procedure of MacMaster and Carmichael [5] was used. Total RNA or poly (A)-rich RNA was denatured by incubation at 50°C for 60 min in 10 mM sodium phosphate buffer pH 7.0 containing 1 M glyoxal and 50% dimethylsulphoxide and was electrophoresed on a 1.0% agarose gel in 10 mM sodium phosphate buffer pH 6.8. After electrophoresis, the RNA was transferred to nitrocellulose paper (GeneScreen Plus, DuPont, Wilmington, USA), deglyoxylated with 50 mM NaOH and hybridized with a cDNA probe labeled with [α-^{32}P]CTP by random primer (Gibco BRL, Gaithersburg, USA). The probes used were as follows: the *Hin*dIII–*Hin*dIII fragment of 2.6K base pairs derived from the plasmid pVLDLR1 [1] carrying rabbit very low density liproprotein receptor cDNA; the *Apa*I–*Apa*I fragment of 1.5K base pairs derived from the plasmid pLDLR11 [6] carrying rabbit liver low density liproprotein receptor cDNA; the *Not*I–*Eco*RV fragment of 1.5K base pairs carrying human β-actin cDNA [7]. Prehybridization was performed at 42°C for 4 h in 50 mM sodium phosphate buffer pH 6.8 containing 50% formamide, 0.75 M NaCl, 75 mM trisodium citrate, 0.02% polyvinyl-pyrrolidon-K90, 0.02% Ficoll 400

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(Pharmacia Fine Chemicals, Uppsala, Sweden), 0.02% bovine serum albumin Fraction V from Sigma Chemical Co., St. Louis, USA, 250 μg/ml sonicated denatured salmon sperm DNA and 0.1% sodium dodecyl sulfate. The hybridization solution contained, in addition, 2 ng/ml ^{32}P-labeled cDNA with a specific activity of 1.5×10^9 counts/min/μg. The hybridization temperature was 42°C for both VLDL receptor cDNA and LDL receptor cDNA. After hybridization for approximately 14 h, the filters were washed at the same temperature for 1 h, blotted, covered with plastic wrap and autoradiographed at −70°C with intensifying screens (Quanta III, DuPont).

3. Results and discussion

In the first series of experiments (Fig. 1), pairs of rabbits were treated with ethinyl estradiol and total RNA preparations derived from the ventricle of the heart of both animals were pooled and subjected to blot hybridization analysis with a rabbit VLDL receptor cDNA probe. Total RNA from pairs given 5.0 mg/kg ethinyl estradiol for 4 days (lane 2 in Fig. 1A) and 8 days (lane 3 in Fig. 1A) contained about 2.5 times and 5 times VLDL receptor mRNA than did control rabbits which were sacrificed at 0 day (lane 1 in Fig. 1A). The filters

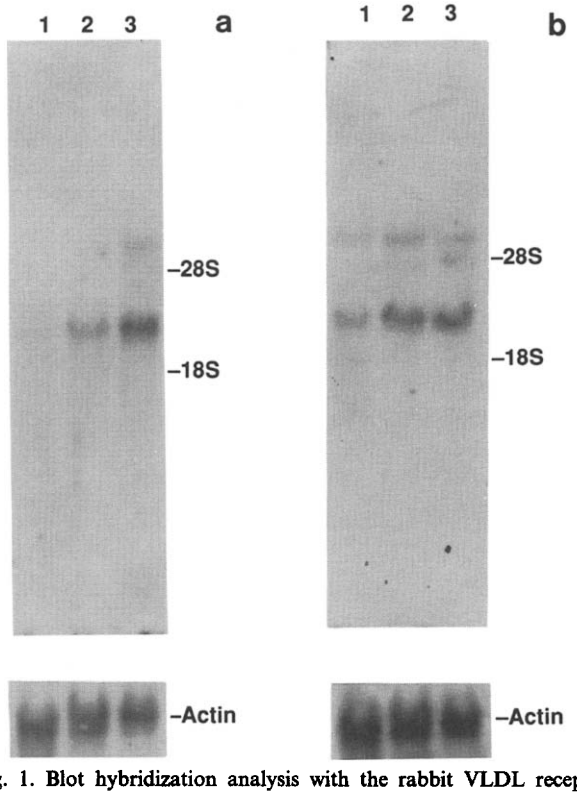

Fig. 1. Blot hybridization analysis with the rabbit VLDL receptor cDNA probe of total RNA from rabbit ventricles and gastrocnemius treated with estradiol at 5.0 mg/kg. Total RNA (20 μg) from rabbit heart ventricles (A) and gastrocnemius musle (B) following electrophoresis on an agarose gel, transfer to a nitrocellulose membrane, and hybridization with rabbit VLDL receptor cDNA(A) and then rehybridized with human β-actin cDNA probe. Lane 1, total RNA from control rabbit; lane 2, total RNA after 4 days of ethinyl estradiol administration; lane 3, 8 days ethinyl estradiol. Markers are 28S ribosomal RNA and 18S ribosomal RNA.

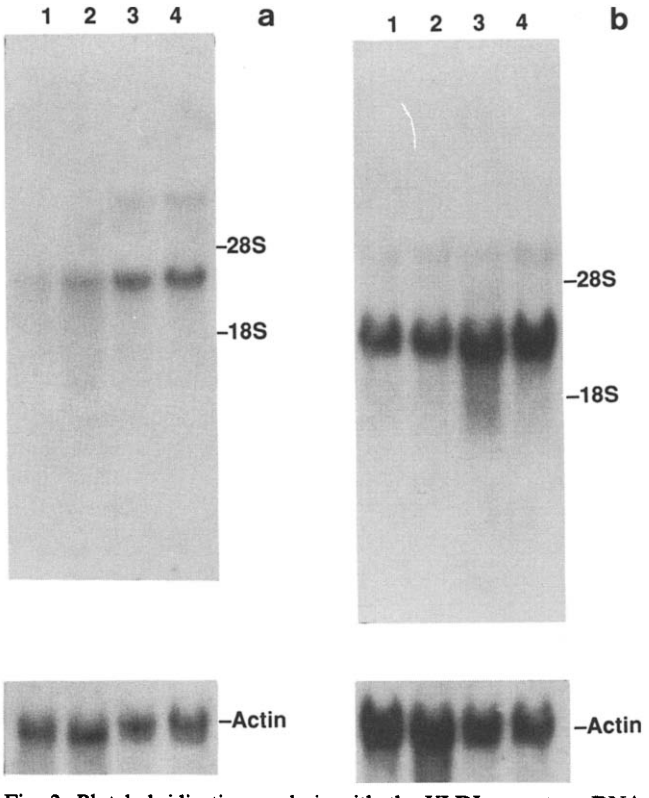

Fig. 2. Blot hybridization analysis with the VLDL receptor cDNA probe of total RNA from rabbit ventricles treated with 2.5 mg/kg or 5.0 kg/mg estradiol. Total RNA (20 mg) from rabbit heart ventricles treated with ethinyl estradiol at 2.5 mg/kg(A) or 5.0 mg/kg(B) were analyzed by same the procedure as in Fig. 1. Lane 1, total RNA from control rabbit; lane 2, 2 days ethinyl estradiol administraion; lane 3, 4 days ethinyl estradiol; lane 4, 6 days ethinyl estradiol. Markers are the same as in Fig. 1.

were rehybridized with a human β-actin cDNA probe. A signal band corresponding to β-actin mRNA was detected in all lanes and there was no significant difference in band density among the lanes. Total RNA from gastrocunemius were also analyzed (Fig. 1B). The level of VLDL receptor mRNA was increased two times with estradiol administration for 4 days (lane 2 in Fig. 1B) and 8 days (lane 3 in Fig. 1B).

In the second series of experiments, rabbits were treated with ethinyl estradiol at 2.5 mg/kg (Fig. 2A) and 5.0 mg/kg (Fig. 2B) for 2, 4, or 6 days. The ventricle and liver derived from each rabbit administered with ethinyl estradiol were analyzed. The results show that administration of ethinyl estradiol gradually increased the level of VLDL receptor mRNA in the rabbit ventricle: in Fig. 2A, VLDL receptor mRNA levels for 2.5 mg/kg over 2 days (lane 2), 4 days (lane 3) and 6 days (lane 4) were scored by densitometry scanning as 321%, 561% and 684% of the control value (lane 1 in Fig. 2A). Rabbits treated at 5.0 mg/kg estradiol, the same dose as in the first experimental series, also showed a large increase in VLDL receptor mRNA content, although at this dose there was no relationship between VLDL receptor

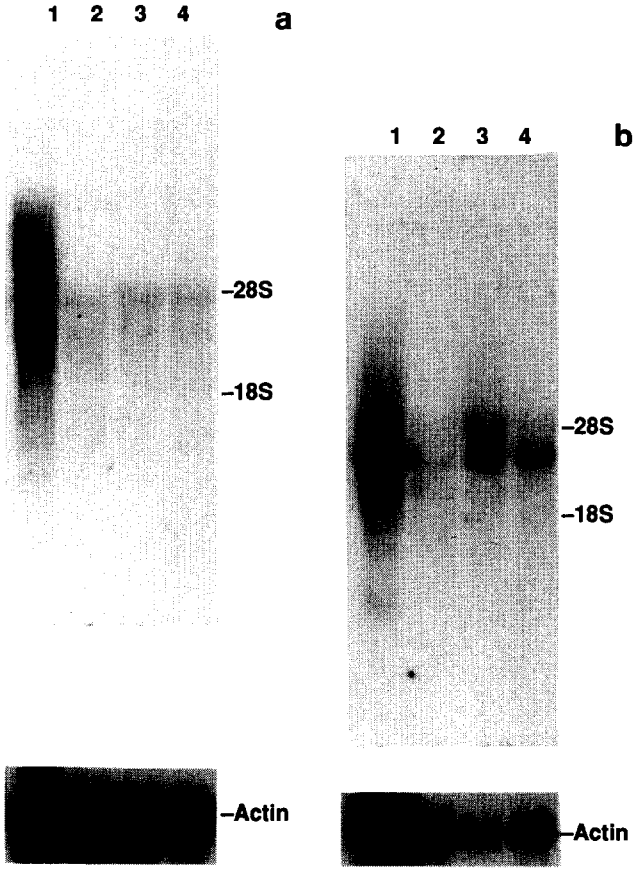

Fig. 3. Blot hybridization analysis with the rabbit LDL receptor cDNA probe of total and poly (A) rich RNA from rabbit ventricle and liver treated with estradiol. Poly(A) RNA (20 μg) (lanes 1) and total RNA (20 μg) (lanes 2, 3 and 4) from rabbit ventricle (A) and liver (B) of rabbits treated with ethinyl estradiol at 2.5 mg/kg were analyzed by RNA blotting with rabbit LDL receptor cDNA probe. Lanes 1 and 2, control rabbit; lane 3, 4 days of estrdiol administration of 2.5 mg/kg ethinyl estradiol; lane 4, 6 days ethinyl estradiol.

mRNA content and time of estradiol exposure (Fig. 2B). Under the same condition, LDL receptor mRNA was not detected in the heart ventricle total RNA preparation (Fig. 3A), although by using 20 μg of poly(A)-rich RNA we could confirm the presence of low levels of LDL receptor mRNA in the heart. It is, therefore, clear that in heart ventricle ethinyl estradiol greatly enhances the expression of VLDL receptor mRNA but not of LDL receptor mRNA.

VLDL receptor mRNA was not detected in the liver even after the administration of estradiol (data not shown) but LDL receptor mRNA is expressed in rabbit livers (Fig. 3B) and its level was increased by the estradiol treatment. This enhanced expression of LDL receptor mRNA in the liver by estradiol is consistent with the previous results [8–11].

The current study has been designed to assess effects of estrogen on the expression of the VLDL receptor gene. Sterol represses the expression of LDL receptor in cultured THP-1 cells, but it does not change the expression of the VLDL receptor gene (unpublished observation). It is reported that pharmacological doses of ethinyl estradiol induce the LDL receptor mRNA and its protein in the rabbit liver [11]. Increased LDL receptors in the liver enhance the catabolism of LDL and results in lower levels of plasma LDL. The precise mechanism of estradiol enhancement on LDL receptor expression is unknown. The 5′-flanking region of the VLDL receptor gene contains several regulatory elements including the half sites for interaction with estrogen receptors [3]. Whether or not the estrogen receptor interacts directly with the above sequence remains to be elucidated by further analyses of the promoter region.

In Watanabe heritable hyperlipidemic (WHHL) rabbit, which is an animal model for the hereditary deficiency of LDL receptor, chylomicron remnant catabolism is not impaired [12]. This finding led to the hypothesis that, in the liver, there is a chyromicron remnant receptor which recognizes triglyceride rich apoE containing lipoprotein particles. Recently, LDL receptor-related protein has emerged as a candidate receptor for apoE containing lipoprotein particles [13]. In contrast, the VLDL receptor was not expressed significantly in the rabbit liver but was abundantly expressed in the heart, muscle and adipose tissue. So far, lipoprotein catabolism in the heart has not been studied intensively. It is thought that VLDL particles are hydrolyzed by lipoprotein lipase (LPL) in the capillary lumen, liberating free fatty acid which then diffuses into muscle or adipose tissue cells. From the stand point of energy supply, the direct entry of triglyceride rich lipoprotein particle into cells via the VLDL receptor appears highly efficient. The VLDL receptor in the heart might therefore be a gate for basal energy supply. Alternatively, this novel lipoprotein receptor might function in the heart when energy demand is increased. To further clarify its function(s), regulation of VLDL receptor expression needs to be studied in pathological conditions, such as cardiac hypertrophy.

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References

[1] Takahashi, S., Kawarabayasi, Y., Nakai, T., Sakai, J. and Yamamoto, T. (1992) Proc. Natl Acad. Sci. USA 89, 9252–9256.
[2] Brown, M.S. and Goldstein, J.L. (1986) Science 232, 34–47.
[3] Sakai, J., Hoshino, A., Takahashi, S., Miura, Y., Ishii, H., Kawarabayasi, Y. and Yamamoto, T. (1994) J. Biol. Chem. 269, 2173–2182.
[4] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[5] MacMaster, G.K. and Carmichael, G.G. (1977) Proc. Natl Acad. Sci. USA 74, 4835–4838.

[6] Yamamoto, T., Bishop, R.W., Brown, M.S., Goldstein, J.L. and Russel, D.W. (1986) Science 232, 1230–1237.
[7] Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H. and Kedes, L. (1983) Mol. Cell. Biol. 3, 787–795.
[8] Kovanen, P.T., Brown, M.S. and Goldstein, J.L. (1979) J. Biol. Chem. 254,11367–11373.
[9] Chao, Y .-S., Windler, E.E., Chen, G.C. and Havel, R.J. (1979) J. Biol. Chem. 254, 11360–11366.
[10] Windler, E.E.T., Kovanen, P.T., Chao, Y.-S., Brown, M.S., Havel, R.J. and Goldstein, J.L. (1980) J. Biol. Chem. 255, 10464–10471.
[11] Ma, P.T., Yamamoto, T., Goldstein, J.L. and Brown, M.S. (1986) Proc. Natl Acad. Sci. USA 83, 792–796.
[12] Kita, T., Goldstein, J.L., Brown, M.S., Watanabe, Y., Hornick, C.A. and Havel, R.J. (1982) Proc. Natl Acad. Sci. USA 79, 3623–3627.
[13] Herz, J., Hamann, U., Rogne, S., Myklebost, O., Gausepohl, H. and Stanlley, K.K. (1988) EMBO J. 7, 4119–4127.