FGF-1 induces expression of LXRα and production of 25-hydroxycholesterol to upregulate the apoE gene in rat astrocytes

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Abstract **Fibroblast growth factor 1 (FGF-1) enhances apolipoprotein E (apoE) expression and apoE-HDL biogenesis in autocrine fashion in astrocytes (Ito, J., Y. Nagayasu, R. Lu, A. Kheirollah, M. Hayashi, and S. Yokoyama. Astrocytes produce and secrete FGF-1, which promotes the production of apoE-HDL in a manner of autocrine action. *J. Lipid Res.* 2005. 46: 679–686) associated with healing of brain injury (Tada,T., J-i. Ito, M. Asai, and S. Yokoyama. Fibroblast growth factor 1 is produced prior to apolipoprotein E in the astrocytes after cryo-injury of mouse brain. *Neurochem. Int.* 2004. 45: 23–30). FGF-1 stimulates mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) to increase cholesterol biosynthesis and phosphatidylinositol 3-OH kinase (PI3K)/Akt to enhance apoE-HDL secretion (Ito, J., Y. Nagayasu, K. Okumura-Noji, R. Lu, T. Nishida, Y. Miura, K. Asai, A. Kheirollah, S. Nakaya, and S. Yokoyama. Mechanism for FGF-1 to regulate biogenesis of apoE-HDL in astrocytes. *J. Lipid Res.* 2007. 48: 2020–2027). We investigated the mechanism for FGF-1 to upregulate apoE transcription. FGF-1 increased apoE and liver X receptor α (LXRα) mRNAs in rat astrocytes. Increase of LXRα mRNA was suppressed by inhibition of the FGF-1 receptor-1 and MEK/ERK but not by inhibition of PI3K/Akt. The increases of apoE mRNA and apoE-HDL secretion were both inhibited by downregulation or inhibition of LXRα, while they were partially suppressed by inhibiting cholesterol biosynthesis. We identified the liver X receptor element responsible for activation of the rat apoE promoter by FGF-1 located between −450 and −320 bp, and the direct repeat 4 (DR4) element in this region (−448 to −433 bp) was responsible for the activation. Chromatin immunoprecipitation analysis supported that FGF-1 enhanced association of LXR with the rat apoE promoter. FGF-1 partially activated the apoE promoter even in the presence of an MEK inhibitor that inhibits the FGF-1-mediated enhancement of cholesterol biosynthesis. On the other hand, FGF-1 induced production of 25-hydroxycholesterol by MEK/ERK as an sterol regulatory element-dependent reaction besides cholesterol biosynthesis. We concluded that FGF-1-induced apoE expression in astrocytes depends on LXRα being mediated by both LXRα expression and an LXRα ligand biosynthesis.**—Lu, R., J. Ito, N. Iwamoto, T. Nishimaki-Mogami, and S. Yokoyama. **FGF-1 induces expression of LXRα and production of 25-hydroxycholesterol to upregulate the apoE gene in rat astrocytes. *J. Lipid Res.* 2009.** 50: **1156–1164.**

Supplementary key words high density lipoprotein • cholesterol • direct repeat 4

Apolipoprotein E (apoE) is a glycoprotein composed of 299 amino acids and plays critical roles in lipid metabolism. While most of apolipoproteins are synthesized and secreted primarily by the liver and intestine, apoE is in addition secreted by other cells outside the enterohepatic axis, such as macrophages and adipocytes (1, 2). ApoE is also synthesized by astrocytes and microglia in the central nervous system (CNS) and forms HDL as a major lipoprotein in CNS (3, 4). CNS is segregated from systemic circulation by the blood brain barrier and prevented from access to plasma lipoproteins, so that HDL generated in CNS is the exclusive lipid transport system among the CNS cells (5). ApoE-HDL plays many key roles in maintaining integrity and regeneration of CNS by mediating intercellular lipid transport. ApoE synthesis in glia cells increases during development and after injury of CNS (6–11).

We demonstrated that absence of apoE delayed healing of cryo-injury of mouse brain, as evidenced by expression

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Abbreviations: apoE, apolipoprotein E; ChIP, chromatin immunoprecipitation; CNS, central nervous system; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FGFR1, FGF receptor 1; LXR, liver X receptor; LXRE, LXR response element; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-OH kinase; siRNA, small interfering RNA; SRE, sterol regulatory element; TK, thymidine kinase.

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of fibroblast growth factor 1 (FGF-1) and subsequent apoE expression in the astrocytes in the peri-injury regions (12). Long-term culture of rat astrocytes induced the increase of apoE synthesis and apoE-HDL production and enhancement of cholesterol biosynthesis, in comparison to the astrocytes prepared by a conventional method of 1-week primary and 1-week secondary culture (13). FGF-1 was highly expressed and released in these long-cultured cells, and their conditioned medium and FGF-1 both stimulated apoE synthesis, production of apoE-HDL, and cholesterol biosynthesis in the conventionally prepared astrocytes (14).

FGF-1 was shown in astrocytes to activate the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signaling pathway for the increase of cholesterol biosynthesis and the phosphatidylinositide 3-OH kinase (PI3K)/Akt pathway to enhance secretion of apoE/apoE-HDL, being mediated by the receptor (15). It also stimulated transcription of the apoE gene mediated by the receptor, but with an unknown pathway (15). To understand the mechanism for FGF-1 to stimulate astrocytes for biogenesis of apoE-HDL in response to brain injury and to expedite its healing process, we further investigated the mechanism for FGF-1 to increase transcription of apoE in astrocytes. FGF-1 was shown to enhance apoE transcription through the increase of interaction of the liver X receptor (LXR)α, a nuclear receptor for oxysterol being involved in sterol homeostasis, with a conserved direct repeat 4 (DR4) sequence in LXR response element (LXRE) present in the apoE promoter. This reaction was mediated by the increase of LXRα expression and by the production of its ligand.

MATERIALS AND METHODS

Cell Culture

Astrocytes were prepared from 17-day fetal brain of Wistar rats according to the method previously described (16). The brain cells were cultured in F-10 medium (GIBCO) containing 10% (v/v) fetal calf serum for 1 week as primary culture and for subsequent 1 week as secondary culture. Fibroblasts of BALB/3T3 mouse were obtained from the RIKEN cell bank. The cells were cultured in DF medium (1:1 mixture of DMEM and Ham's F12 medium) with 10% (v/v) fetal calf serum.

RNA Isolation and real-time PCR analysis

Total RNA was isolated from the cells using an ISOGEN kit (Nippon Gene). Real-time PCR analysis was performed on an ABI PRISM 7700 sequence detection system using target-specific probes. Those for apoE and LXRα were described elsewhere (14, 17). For HMG-CoA reductase, SREBP2, cholesterol 24-hydroxylase (CYP46A1), and cholesterol 25-hydroxylase, the probes chosen were 5′-TGCTGCTTTGGCTGTATGTC-3′ and 5′-TGAGCGTGAACAAGAACCAG-3′, 5′-CCAAGAAGAAGGCAGGTGAC-3′ and 5′-GGACCCGCTCTACTTCAGTG-3′, 5′-GTGACTATGGGCGCTGGTAT-3′ and 5′-ATCAGCTGCTCTGCCTTCTC-3′, and 5′-TAGCCTTCTTGGATGTGCTG-3′and 5′-GTGAGTGGACCACGGAAAGT-3′, respectively.

Western blot analysis

Cell lysates or media concentrates (concentrated using the BCA Protein Assay Kit; Pierce) were subjected to 10% SDS-PAGE (50 μg protein/lane) and then transferred to a polyvinylidene difluoride membrane. Bands of apoE were visualized by using the specific antibody (Santa Cruz Biotechnology). Intensity of photoimage was digitalized by scanning using an EPSON GT-X700 and analyzed with Adobe Photoshop software for quantification.

RNA interference

Small interfering RNA (siRNA) specific primers for rat LXRα and a scramble control were obtained from Invitrogen and transfected into rat primary astrocytes using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

Construction of luciferase reporter genes and luciferaase assay

The promoter region of rat apoE gene was prepared by PCR using rat normal liver genomic DNA (18) as a template using primers tailed with *Kpn*I and *Xho*I (New England Biolabs). Each PCR product was ligated into *Kpn*I/*Xho*I sites of PGVB Basic vector and confirmed by sequencing. Six reporter gene constructs were assembled with the segments of the apoE promoter from −690, −600, −450, −320, −200, and −135 bp to +9 bp. To obtain a reporter construct with mutation of apoE DR4 element (DR4mu), site-directed mutagenesis was performed to introduce the DR4 at −448 to −433 bp of the −690 to +9 reporter gene using a Quick Change II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and the respective primers, DR4 of forward: 5′-CCGGGGATGGGGAGttaaCACCGTGGCAGAGGAATCACTA-3′, reverse: 5′-TAGTGATTCCTCTGCCACGGTGttaaCTCCCCATCCCCGG-3′ (lowercase letters indicate mutated nucleotides). The plasmid for expression of LXRα and the reporter plasmid containing four tandem repeats of LXREs upstream of the thymidine kinase (TK) promoter and its mutant, LXRE-TK and LXREmut-TK, the promoter plasmid containing sterol regulatory element (SRE), were prepared as previously described (19, 20). 3T3 cells were grown to 60–70% confluence in 24-well plates, transfected with 1 μg of plasmid DNA including 0.2 μg LXRα expression vector, 0.77 μg reporter gene construct, and 30 ng hRluc-TK (Promega; for normalization) using Lipofectamine 2000 (Invitrogen). The luciferase activity was measured using a Dual-Luciferase Reporter System (Promega).

Chromatin immunoprecipitation analysis

Chromatin immunoprecipitation (ChIP) assay was conducted as previously described (20). Briefly, rat primary astrocytes were fixed with 1% formaldehyde. Cross-linked adducts were resuspended and sonicated to yield DNA fragments of 200 to 1,200 bp. Immunoprecipitation was performed using anti-LXRα antibody (Perceus Proteomics). Mouse normal IgG (Santa Cruz Biotechnology) was used as a control for nonspecific binding. Protein-bound, immunoprecipitated DNA was reverse cross-linked at 65°C overnight and then purified using a PCR purification Kit (Sigma-Aldrich). Ten microliters from a 50-μl DNA extraction volume was used for PCR amplification (33 cycles). The set of primers forward, 5′-CAGAGCTAACAAGTAACACA-3′, and reverse, 5′-AAAAGGGCTGGAGGCTTAAA-3′, was used to amplify the region on the promoter of the apoE gene.

Analysis of cholesterol biosynthesis and oxysterol production

Cholesterol biosynthesis in astrocytes was estimated by incubating astrocytes with ^{3}H-acetate as described elsewhere (15). Lipid was extracted from the cells and analyzed by TLC. For evaluation of production of 25-hydroxycholesterol, the cells were labeled with ^{14}C-cholesterol conjugated with 2% cyclodextrin for 30 min at 37°C, washed with PBS three times, and incubated in 0.1% BSA-F-10 in the presence of FGF-1, U0126, or compactin for 16 h.

Lipid was extracted and analyzed by TLC to detect the count in 25-hydroxycholesterol. Efficacy of the lipid extraction procedure (16) was 88 ± 5% estimated by the recovery of ^{14}C-cholesterol exogenously added.

Other Reagents

FGF-1, TO901317 (an LXR agonist), PD173074, LY294002, and U1026 (inhibitors of the FGF receptor 1 (FGFR1), PI3K, and MEK1/2, respectively) were all purchased from Sigma-Aldrich. SB203580 and SP600125, inhibitors of P38 mitogen-activated protein kinase and stress-activated protein kinase/c-Jun NH2-terminal kinase, respectively, were also obtained from Sigma-Aldrich.

RESULTS

Astrocytes were stimulated by FGF-1 at a confluent stage, and the levels of mRNA were measured for LXRα and apoE in time-dependent manners (**Fig. 1A**). The both messages increased by the FGF-1 treatment up to 12 h in parallel, by 3.5-fold for LXRα and by 7.5-fold for apoE mRNA. LXRα mRNA further increased at least for 24 h by the incubation, while apoE mRNA reached plateau at 12 h. FGF-1 enhanced expression of ABCA1 mRNA being consistent with the increase of LXRα but did not influence expression of LXRβ (Fig. 1B). Fig. 1C shows the increases of apoE mRNA and LXRα mRNA expressions by FGF-1 and their almost complete suppression by an FGFR1 inhibitor PD173074. An MEK/ERK pathway inhibitor, U0126, partially suppressed expression of apoE and LXRα mRNAs. The inhibition beyond the level of control without exogenous FGF-1 may indicate the presence of the basal autocrine activation by endogenous FGF-1 (14). A PI3K/Akt pathway inhibitor LY294002 that inhibits apoE secretion (15) did not influence the expression of either mRNA.

As the apoE gene transcription was reportedly regulated by LXRs (21, 22), we examined whether the FGF-1-induced expression of apoE depends on LXR. Rat astrocytes were transfected with LXRα siRNA to reduce its mRNA expression by 80% and examined for the reactivity of apoE expression by FGF-1. Expression of apoE was reduced by the siRNA treatment estimated as the mRNA and protein level, regardless of the presence of FGF-1, and this was reflected by the decrease in cellular and secreted apoE protein (**Fig. 2A**). Dependency of the FGF-1-induced apoE expression on LXR was also demonstrated by inhibition of LXR by arachidonic acid, a competitive but not highly specific inhibitor of LXRα (23), shown as a decrease of cellular and secreted apoE (Fig. 2B). LXRα is activated by oxysterol that generally increases in parallel with cellular cholesterol, so that the effect of inhibition of cholesterol biosynthesis was examined. The cells were treated with compactin, an HMG-CoA reductase inhibitor, and the FGF-1-induced increase of LXRα mRNA was decreased by overall 62% (85% of the increase by exogenous FGF-1) and that of apoE mRNA was by 74% (**Fig. 3**). Thus, upregulation of the apoE gene transcription in astrocytes is associated with the increase of LXRα, both being regulated by cellular sterol biosynthesis.

To investigate molecular mechanism for FGF-1 to upregulate apoE expression, the promoter of the apoE gene

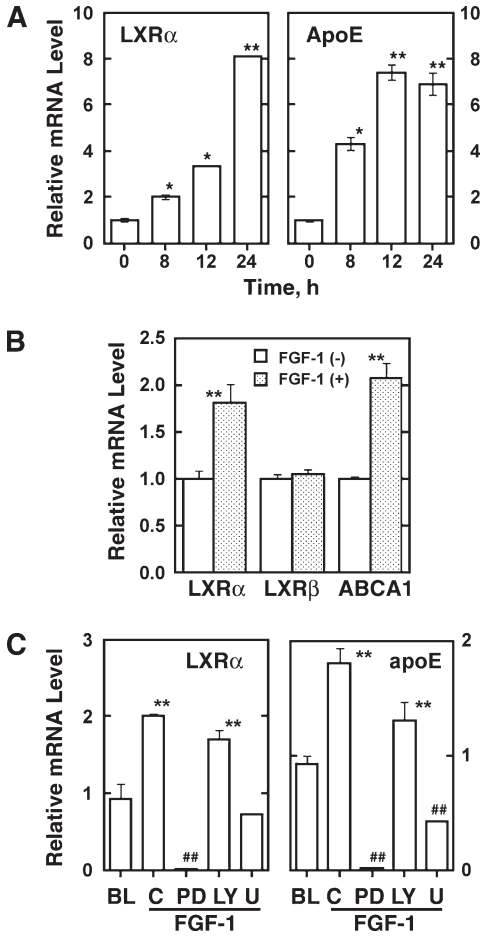

Fig. 1. A: Increase of expression of LXRα and apoE by FGF-1 in rat astrocytes. Primary astrocytes at a confluent stage were treated with 50 ng/ml FGF-1 for the indicated times, and mRNA of LXRα and apoE was determined by real time-PCR. The data are standardized for β-actin and expressed as relative increase to the cells incubated without FGF-1 as controls. B: Effects of FGF-1 on expression of ABCA1 and LXRβ. Experiments were performed as described above with incubation of the cells with FGF-1 for 8 h. C: Induction of mRNA of LXRα and apoE in rat astrocytes. Rat astrocytes in primary culture were treated with FGF-1 in the presence of 10 μM PD173074 (PD), LY294002 (LY), U1026 (U), inhibitors of FGFR1, PI3K, and MEK, respectively. The messages for LXRα and apoE were quantified by real-time PCR as described as above. Data represent the average ± SD of three measurements (some SD values may be too small to appear in the graph). * $P < 0.05$ and ** $P < 0.01$ increase from control. ## $P < 0.01$ decrease from control.

was analyzed by using luciferase reporter assay. Six reporter genes were constructed to find a specific region(s) responsible for the FGF-1 induced activation, between −690 and +9 bp of the apoE promoter, −600 and 9 bp, −450 and +9 bp, −320and 9 bp, −200 bp and 9 bp, and −135 bp and +9 bp (**Fig. 4A**). The activity of the reporter genes was examined for the response to the FGF-1 treatment as well as to an LXR agonist, TO901317, in the transiently transfected 3T3 cells under overexpression of LXRα. As shown in Fig. 4B, FGF-1 and TO901317 activated the reporter genes as far as they contain the region −450 bp or its upstream, and this response was lost with the gene of −320 bp. We thus assumed

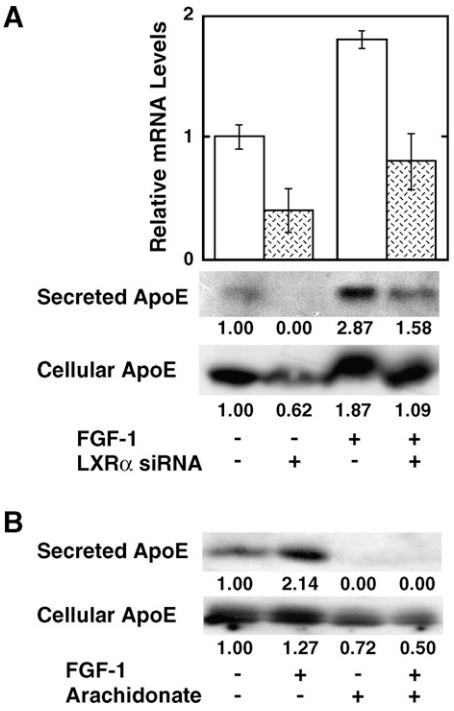

Fig. 2. Effects of inhibition of LXRα expression or of its activation on expression of apoE mRNA and its secretion in astrocytes. A: Rat primary astrocytes were transfected with 20 nM LXRα siRNA and control siRNA as described in the text to suppress its expression. After the 72-h transfection, the cells were treated with 50 ng/ml FGF-1 for 16 h. apoE mRNA and cellular apoE protein at 16 h and apoE secreted into the medium during 16 h were determined by real-time PCR and by Western blotting, respectively. The data are expressed as relative to those by a control scrambled siRNA. B: Rat astrocytes were incubated with 100 μM arachidonate for 16 h to inhibit LXR activity before the treatment with 50 ng/ml FGF-1 for 16 h in the presence of arachidonate. Cellular and secreted apoE were estimated by Western blotting. Data represents the average ± SD of three measurements. * $P < 0.05$ to siRNA (−). Numbers indicate density of the bands relative to control.

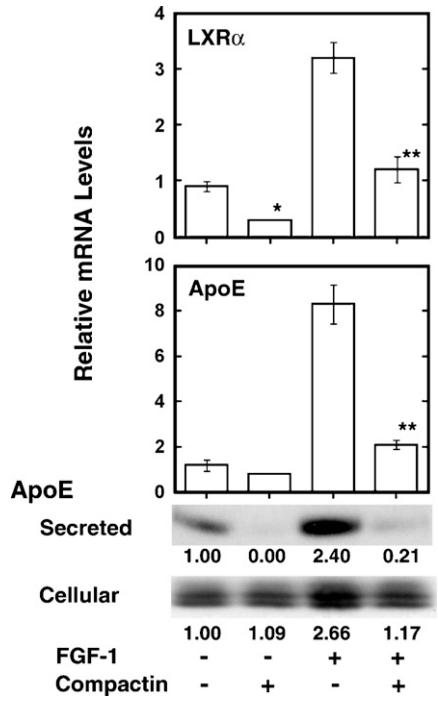

Fig. 3. Effects of inhibition of cholesterol biosynthesis on the increase of expression of LXRα and apoE, as well as on secretion of apoE. Rat primary astrocytes were pretreated with 5 μM compactin for 2 h, and the cells were treated with 50 ng/ml FGF-1 for 16 h. Expression of mRNA of apoE and LXRα was determined by real-time PCR, and cellular and secreted apoE were analyzed by Western blotting. Data represent the average ± SD of three measurements. * $P < 0.05$ and ** $P < 0.01$ to compactin (−). Numbers indicate density of the bands relative to control.

that the region between −450 and −320 bp is responsible for activation by LXR and FGF-1. DR4 sequence was identified as AGTTCACCGTGGCAGA (−448 to −433 bp) in this region, so that mutation was introduced to this sequence as TTAACACCGTGGCAGA of the −600 to +9 bp construct (LXREmut) (Fig. 4A), and its activity was examined. Responses of the reporter genes to FGF-1 and to TO901317 both disappeared by introducing this mutation (Fig. 4C). To confirm that this sequence is generally responsible for activation by FGF-1, the reporter genes were constructed with the heterologous promoter of TK that contains the same DR4 sequence. The promoter was activated by FGF-1, and activation was abolished by introducing mutation into the DR4 in this assay system (Fig. 4D). Treatment of the cells of the reporter assay system with an LXR antagonist, arachidonate, decreased the activation by FGF-1 (Fig. 4E) and so did compactin (data not shown). We concluded from these results that one LXRE exit of −448 to −433 bp of the apoE promoter is responsible for increase of the apoE gene transcription by FGF-1 being dependent on LXR.

To confirm that FGF-1 enhances association of LXR with LXRE of the endogenous apoE promoter in astrocytes, ChIP assay was performed in rat primary astrocytes using specific antibody against rat LXRα (**Fig. 5**). Increase of the association was demonstrated by an LXR agonist TO901317 as well as by FGF-1.

The experiments above demonstrated that FGF-1 activates LXR to enhance expression of the apoE gene through its interaction with the LXRE. **Figure 6** demonstrates production of LXR ligands by FGF-1. Cholesterol biosynthesis is increased by FGF-1 (Fig. 6A) in association with increase of expression of the HMG-CoA reductase gene (Fig. 6B). This increase seems to be mediated by activation of the SRE and SRE binding protein system because FGF-1 activated the SRE-luciferase assay system (Fig. 6C). Cholesterol 25-hydroxylase is known to be activated by the SRE system (24), and its expression was indeed increased by FGF-1 and by compactin to a less extent, while expression of cholesterol 24-hydroxylase that is regulated by LXR (24) was not significantly increased by FGF-1 (Fig. 6D, E). Consequently, production of 25-hydroxycholesterol was increased by FGF-1 in astrocytes (Fig. 6F). These effects of FGF-1 were all blocked by an inhibitor of the MEK/ERK pathway, U0126. Thus, FGF-1 at least increases a ligand of LXR, 25-hydroxycholesterol, for the enhancement of the apoE gene expression.

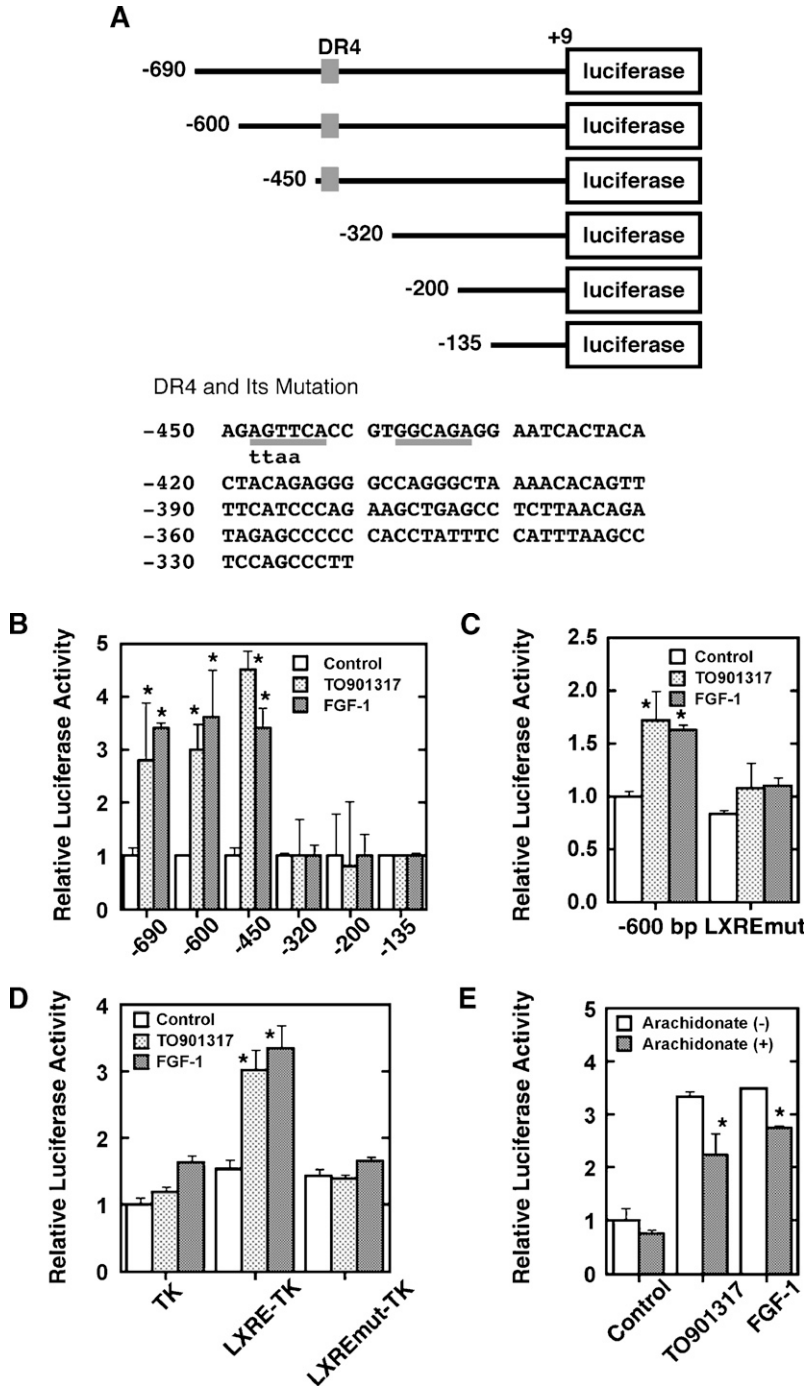

Fig. 4. The results of the reporter gene assays to identify the element responsible for activation by FGF-1 in the apoE promoter or in the heterologous TK promoter that contains 4 × DR4s. The reporter gene constructs were cotransfected with the expression vector for LXRα in 3T3 cells. After 24-h transfection, the cells were incubated with 50 ng/ml FGF-1 or 5 μM T0901317, an agonist for LXRα for 16 h. A: Diagram of the reporter gene constructs for the rat apoE promoter. Numbers indicate of the nucleotide positions, and location of DR4 sequence is indicated by gray boxes. Nucleotide sequence of the segment that contains DR4 and mutations introduced are indicated as lowercase letters for the corresponding nucleotide positions. B: The results with the apoE promoter constructs with various lengths as listed in A. The baseline activity of each reporter gene was 1.22 for −600 bp, 0.72 for −450 bp, 1.44 for −320 bp, 1.67 for −200 bp, and 1.46 for −135 bp, relative to that of −600 bp, respectively. C: The apoE promoter construct of −600 bp and its DR4 mutant (LXREmut) defined in Fig. 4A were used in luciferase assay with TO901317 and FGF-1. D: The results with the TK promoter and its mutant genes, by activation with TO901317 and FGF-1. E: The effects of an LXRα inhibitor, arachidonate, on activation by TO901317 and FGF-1 of the apoE promoter construct of −600 bp. Incubation was carried out as described in the text. Data represent the average ± SD of three measurements/samples. * $P < 0.01$ to the respective control.

We previously demonstrated that FGF-1 enhanced the apoE gene transcription even in the conditions that either the MEK/ERK or PI3K/Akt pathway was inhibited, for enhancement of cholesterol biosynthesis or activation of apoE secretion, respectively (15). The response of the apoE gene promoter to FGF-1 was therefore examined in the presence of those inhibitors to evaluate whether activation of the apoE gene transcription is independent of the related signaling pathways.

The response of the reporter genes to FGF-1 was examined in the presence of an FGFR1 inhibitor, PD173074, a PI3K/Akt pathway inhibitor, LY294002, or an MEK/ERK pathway inhibitor, U0126. The effect of FGF-1 on expression of the gene of −450 bp was abolished by inhibition of FGFR1. Inhibition of the PI3K/Akt pathway did not influence the affect of FGF-1. On the other hand, inhibition of the MEK/ERK pathway by U0126 partially suppressed the effect of FGF-1 (**Fig. 7A**) in the concentration for complete suppression of cholesterol biosynthesis (data not shown), being consistent with the findings in Fig. 1C that induction of LXRα mRNA by FGF-1 was not influenced by inhibition of either signaling pathway. These responses disappeared when the segment between −450 and −320 bp was deleted (Fig. 7B). Thus, we concluded that enhancement of the

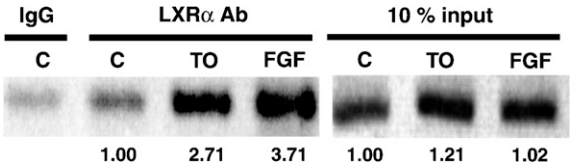

Fig. 5. ChIP analysis for LXRα binding to the apoE promoter. Rat primary culture astrocytes were treated with TO901317 or FGF-1. ChIP analysis was performed with an anti-LXRα antibody and control normal mouse IgG. Primers specific to the LXRE-containing regions of apoE promoter were used for PCR analysis as described in the text. C, control; TO, TO901317; FGF, FGF-1. Numbers indicate density of the bands relative to control.

apoE gene transcription by FGF-1 is dependent on LXRα. The enhancement of the apoE gene transcription seems partially induced by the increase of biosynthesis of the LXRα ligand by FGF-1, but a substantial part may be dependent on the increase of the LXRα expression.

DISCUSSION

apoE is the major endogenous apolipoprotein in CNS, synthesized and secreted by astrocytes and microglias to form apoE-HDL (5). Production of apoE and perhaps apoE-HDL increases in response to acute and chronic damage of CNS, and this seems to play a role in regeneration of nerve cells and healing of the injury. Therefore, it is important to understand the background molecular mechanism for this reaction to understand the process of the recovery of the brain damage. We discovered that apoE-HDL production is stimulated by FGF-1 in astrocytes by an autocrine mechanism and helps the healing process of the brain cryo-injury (12–14). FGF-1 was shown to activate the MEK/ERK signaling pathway to stimulate cholesterol biosynthesis and the PI3K/Ark pathway for enhancement of apoE-HDL secretion (15). It also increases transcription of the apoE gene, but involvement of these pathways is unclear in this reaction (15).

Upregulation of the apoE gene expression was found associated with cellular cholesterol content (25), but the background molecular mechanism for this regulation has not been identified. Two enhancers were identified in the downstream of the human apoE gene, multienhancer 1 and multienhancer 2, that are required for upregulation by sterol and directed macrophage- and adipose-specific expression in transgenic mice (26). The nuclear receptors LXRα and LXRβ and their oxysterol ligands were found to regulate the apoE gene expression by associating LXRE in these enhancers in both macrophages and adipose tissues (21). Thus, the LXR/RXR system is thought to be the major regulatory pathway for apoE gene expression.

LXRs are expressed in CNS (22), and genetic defect of these receptors results in various CNS disorders, such as lipid accumulation, astrocyte proliferation, and disorganization of myelin sheath (27). The LXR/RXR system has also been shown to regulate the apoE gene expression in

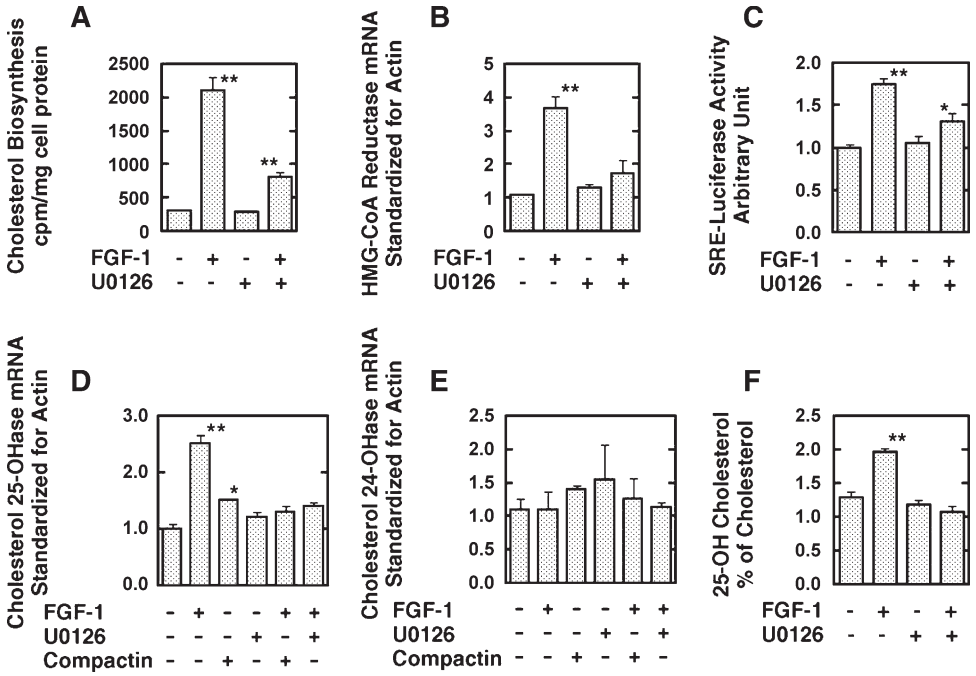

Fig. 6. The effects of FGF-1 to increase cholesterol biosynthesis and production of 25-hydroxycholesterol. A: The effect of FGF-1 and of an inhibitor of the MEK/ERK pathway, U0126. B: Increase of HMG-CoA reductase message and its inhibition by U0126. C: Increase of the SRE reporter gene activity by FGF-1. D: Increase of cholesterol 25-hydroxylase mRNA by FGF-1. The increase by compactin was significant. E: The effects of FGF-1 on cholesterol 24-hydroxylase mRNA. F: Production of 25-hydroxycholesterol. ** $P <$ 0.01 and *$P < 0.05$, respectively.

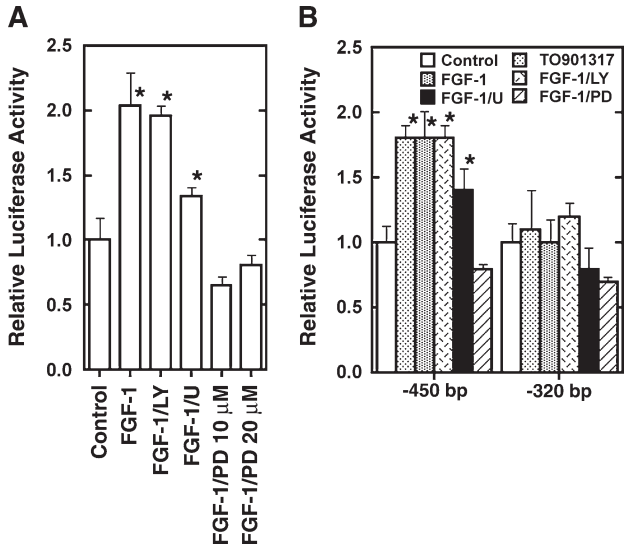

Fig. 7. The effect of inhibitors for FGFR1, the MEK/ERK pathway, and the PI3K/Akt pathway on the reactions induced by 50 ng/ml FGF-1. A: The effects of the inhibitors on the reporter gene expression. The promoter construct of 450 bp was used to investigate contribution of FGFR1 and signaling pathways of MEK/ERK and PI3K to the FGF-1-induced activation of the apoE promoter. The cells were incubated with FGF-1 in the presence of the inhibitors of PI3K, MEK, and FGFR1, as 10 μM of LY294002 (LY) and U1026 (U), and 10 and 20 μM of PD173074 (PD), respectively. B: The effect of the inhibitors on the reporter gene expression. The promoter constructs of 450 and 320 bp were used to confirm that LXRE is responsible for the FGF-1-induced activity and its inhibition. Concentration of TO901317 was 5 μM, and that of the inhibitors was same as above. Data represent the average ± SD of three measurements. * $P < 0.05$ to the respective control.

CNS and to be involved in regulation of cholesterol homeostasis there (28). An LXR ligand, 24(*S*)-hydroxycholesterol and its agonist TO901317, induce LXR-dependent genes, such as apoE, ABCA1, and ABCG1 in astrocytes in vitro (28, 29), but the effect of the latter compound in vivo on apoE expression may not be consistent (30, 31).

FGF-1 is a potent mitogen and growth stimulator that belongs to a family of polypeptide growth factors (32) and stimulates cells through the high affinity tyrosine kinase-linked FGF receptors (FGFR1-4) for their growth, differentiation, and inflammatory reactions (33–35). However, action and behavior of FGF-1 may not be those of typical cytokines that are secreted and react with target cells by inducing intracellular signals. It does not have a signal peptide, synthesized and located in cytosol (36), so that its release from cells is mediated by an unknown mechanism (37). It has a nuclear translocational sequence, and deletion of this domain results in loss of stimulation of DNA synthesis but not induction of signals (38). The effect of FGF-1 on transcription of the apoE gene apparently depends on FGFR-1 and LXR but not on stimulation of the PI3K/Akt pathway and partially on the MEK/ERK pathway to stimulate sterol biosynthesis. Thus, FGF-1 may act to stimulate the LXRα transcriptional activity by direct action of FGF-1 if it could be internalized by FGFR1.

In this article, we investigated the mechanism for FGF-1 to activate expression of the apoE gene. We demonstrated that the promoter of rat apoE gene contains DR4 sequence at the position of −448 to −433 bp, which is responsible for LXRα binding and transcriptional activation of the gene by FGF-1. This DR4 element had homology to the apoE promoter sequence −2590 to −2572 bp of human by 62.5%, and −1439 to −1424 bp and −405 to 389 bp of mouse by 66 and 82%. Functions of these segments are unknown for regulation of the apoE gene. On the other hand, the enhancer DR4 sequence reported by other authors did not match the promoter sequence in this work (21). One multienhancer having DR4 was identified in association with the mouse apoE gene at the corresponding position to human multienhancer 1 with respect to its position (39), and analysis of the rat apoE gene revealed the presence of multienhancer with DR4 in the similar region. Contribution of this DR4 to regulation of the rat apoE gene should be investigated.

It was also demonstrated that transcriptional activation of the apoE gene apparently requires basic level of cholesterol biosynthesis activity in the cells, and inhibition of the increase of cholesterol biosynthesis by FGF-1 may result in its partial decrease, showing that activation of LXR perhaps by oxysterol is a part of the mechanism for this FGF-induced reaction. This view was strongly supported by the findings that FGF-1 induced expression of cholesterol 25-hydroxylase to produce 25-hydroxycholesterol. It is interesting that FGF-1 seems to activate the SRE binding protein pathways to increase cholesterol biosynthesis and therefore activate cholesterol 25-hydroxylase but not cholesterol 24-hydroxylase (24). The effects of compactin on these enzymes also seem consistent with this mechanism. The increase of 25-hydroxycholesterol should activate LXRα and increase apoE transcription. It is interesting that cholesterol 24-hydroxylase transcription was not activated despite the LXR activation, perhaps being consistent with the findings that this enzyme is expressed predominantly in neurons in the brain (40, 41).

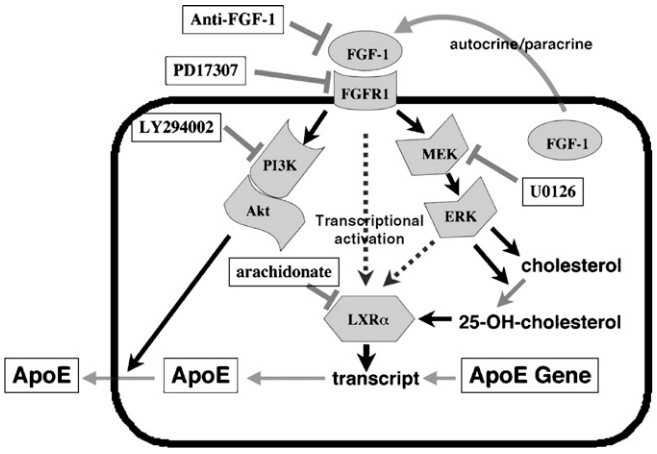

Fig. 8. A schematic model for mechanism by FGF-1 to increase production of apoE-HDL in rat astrocytes. The results from the references (14, 15) and those of this study are summarized.

Since LXRα was shown autoregulated in induction of apoE in mouse adipose tissue (42), the increase of LXRα by FGF-1 can also be through the same mechanism in rat astrocytes. However, FGF-1 also induces expression of the apoE gene apparently in a manner independent of stimulation of cholesterol biosynthesis (15), and this may be consistent with the current result that induction by FGF-1 of the LXRα gene partially remains after inhibition of the MEK/ERK pathway or cholesterol biosynthesis. Further investigation is required to elucidate detail of the mechanism for this cholesterol-independent pathway. It also remains unsolved how FGF-1 enhances apoE secretion by using the PI3K/Akt pathway (15). The results of this article together with our previous works (14, 15) are summarized in **Fig. 8**. Although human apoE gene is also upregulated through LXRs, it has not directly been demonstrated that FGF-1 plays a similar role in acute regulation of apoE-HDL production in the human brain. Studies should be conducted in human astrocytes in appropriate experimental systems.

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