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mRNA for low density lipoprotein receptor in brain and spinal cord of immature and mature rabbits

(myelination/cholesterol homeostasis/3-hydroxy-3-methylglutaryl-coenzyme A synthase/WHHL rabbits)

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ABSTRACT **Hybridization studies with [^{32}P]cDNA probes revealed detectable amounts of mRNA for the low density lipoprotein (LDL) receptor in the central nervous system (CNS) of rabbits. mRNA levels were highest in the medulla/pons and spinal cord, which were the most heavily myelinated regions that were studied. Lower, but detectable levels were present in cerebral cortex, hypothalamus, thalamus, midbrain, and cerebellum. In the medulla/pons and spinal cord, the levels of receptor mRNA were in a range comparable to that detected in the liver. The levels of receptor mRNA in whole brain were constant from 3 days of age to adulthood and, thus, did not vary in proportion to the rate of myelin synthesis. LDL receptor mRNA in the CNS was produced by the same gene that produced the liver and adrenal mRNA as revealed by the demonstration of a deletion in the neural mRNA of Watanabe-heritable hyperlipidemic (WHHL) rabbits identical to the deletion in the LDL receptor gene of these mutant animals. Using antibodies directed against the bovine LDL receptor, we showed that LDL receptor protein is present in the medulla/pons of adult cows. The cell types that express LDL receptors in the CNS and the functions of these receptors are unknown.**

The low density lipoprotein (LDL) receptor, a cell surface glycoprotein, mediates the cellular uptake and degradation of lipoproteins that contain apolipoprotein (apo) B-100 or apoE (1). The receptor removes cholesterol-carrying lipoproteins from plasma and delivers cholesterol to cells. The majority of the LDL receptors in the body are expressed in the liver. Receptors are also abundant in the adrenal gland and the ovary. Other tissues have much smaller numbers of LDL receptors in the resting state, but they are able to increase receptor production when their demand for cholesterol increases, as occurs in phytohemagglutinin-stimulated lymphocytes (2).

LDL receptors have not been studied previously in the central nervous system (CNS), a tissue that is extremely rich in cholesterol. Most of the cholesterol in the CNS of mammals is believed to be synthesized within the CNS itself, where it functions as an essential component of myelin (3). The rate of cholesterol synthesis in rabbits (4), mice (5), and rats (3) is high during the first 30 days of life, when myelin is being deposited in large amounts, and declines thereafter. Not all brain cholesterol is synthesized locally, however; a slow uptake of cholesterol from plasma into the brain occurs throughout life (6). Moreover, cholesterol leaves the brain of rats at a rate of about 0.3% per day (7). These studies suggest that there is a mechanism for exchange of cholesterol between CNS and blood. Whether plasma lipoproteins are involved in this exchange is not known. The cerebrospinal fluid of humans has been shown to contain small amounts of plasma lipoproteins, including apoE, apoC-II, apoC-III, and apoA-I. ApoB was not detected (8).

A potential role for plasma lipoproteins in the CNS has been disclosed recently through experiments of Elshourbagy *et al.* (9) and Boyles *et al.* (10), who demonstrated high levels of mRNA for apoE in the brain and high levels of immunodetectable apoE in the secretory apparatus of astrocytes throughout the brain. The production and secretion of apoE in the brain raises the question as to whether cells in the CNS possess LDL receptors, and whether this production varies with the course of myelination. In the current experiments, we have studied this problem by measuring the amount of mRNA for the LDL receptor in brain and spinal cord of immature and mature rabbits. We have found low but detectable levels of expression that do not appear to vary with age.

MATERIALS AND METHODS

Animals. Male New Zealand White rabbits (4–5 kg) were obtained from Hickory Hill Rabbitry (Flint, TX) and were fed Purina Certified Rabbit Chow. Mature rabbits were exposed to 12 hr of light (6 a.m. to 6 p.m.) and 12 hr of darkness (6 p.m. to 6 a.m.) daily for at least 1 week prior to use and were sacrificed between 10 a.m. and noon. The animals were not fasted. Younger rabbits were obtained from the supplier on the day of each experiment. The animals were not fasted, and most had milk in their stomachs. Male Watanabe-heritable hyperlipidemic (WHHL) rabbits (4–5 kg) were raised in Dallas (12). Bovine tissues were obtained from a local slaughterhouse.

DNA Probes. Uniformly ^{32}P-labeled single-stranded cDNA probes (used for experiments in Figs. 1, 3, and 4 and Table 1) were prepared by primer extension from phage M13 templates (11). For LDL receptor mRNA analysis, a mixture of four rabbit cDNA probes corresponding to nucleotides 717–993, 1176–1497, 1409–2173, and 1378–2445 of the rabbit LDL receptor cDNA-containing plasmid pLDLR11 (12) were used. For hydroxymethylglutaryl-coenzyme A (HMG-CoA) synthase mRNA analysis, a hamster cDNA probe corresponding to the *Pst* I–*Pst* I fragment encompassing nucleotides −35 to 401 of the plasmid p53k-312 (13) was used. A human β-actin cDNA phage M13 clone was prepared as described (14). The specific activity of the probes was $>10^9$ cpm/μg. The probe used for S1 nuclease analysis in Fig. 2 was prepared as described (12).

Blot Hybridization of RNA. Total RNA was isolated from rabbit liver and brain by the guanidinium isothiocyanate/CsCl method (15). RNA was quantified by reaction with orcinol (16) and by UV absorbance and was found to be free

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Abbreviations: apo, apolipoprotein; β-VLDL, β-migrating very low density lipoprotein; CNS, central nervous system; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; WHHL rabbit, Watanabe-heritable hyperlipidemic rabbit.

of contaminating DNA and protein. Total RNA was denatured with glyoxal, size-fractionated on 1.5% agarose gels containing 40 mM 3-(*N*-morpholino)propanesulfonic acid (pH 7.0), transferred to nylon membranes (Zeta-Probe, Bio-Rad) in 20× NaCl/Cit (1× NaCl/Cit = 150 mM NaCl/15 mM sodium citrate), baked, boiled in 20 mM Tris·HCl (pH 8), and prehybridized as described (17). Hybridization with single-stranded ^{32}P-labeled probes was carried out at 42°C for 12–16 hr in 50% (vol/vol) formamide containing 5× Denhardt's solution (1× = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 5× SSPE (1× SSPE = 150 mM NaCl/10 mM NaH_2PO_4/1 mM EDTA, pH 7.4), 1% $NaDodSO_4$, and 100 μg of denatured salmon sperm DNA, 1 μg of poly(adenylic acid), and 1×10^6 cpm of [^{32}P]DNA probe per ml. The membranes were then washed as described in the figure legends.

Slot-Blot Hybridization of RNA. Total RNA (200 μg in 0.4 ml of sterile diethyl pyrocarbonate-treated H_2O) was added to 0.24 ml of 20× NaCl/Cit and 0.16 ml of 37% formaldehyde (Fisher no. F-79) and incubated for 15 min at 60°C. Serial dilutions were made in 10× NaCl/Cit, and 40-μl samples were loaded into the wells of a 72-well Minifold II apparatus (Schleicher & Schuell) and blotted onto a nitrocellulose sheet (Schleicher & Schuell, BA 85; 0.45 μM) as described (18). No hybridization signal was observed from a yeast tRNA control included on each filter.

Immunoblotting and Ligand Blotting. Membranes from bovine liver and brain (whole brain or medulla and pons) were prepared as described (19) except that the following protease inhibitors were present during tissue homogenization and all subsequent procedures: 1 mM phenylmethanesulfonyl fluoride, 1 mM *o,p*-phenanthroline, 0.1 mM leupeptin, and 1 μg of pepstatin and 0.5 μg of aprotinin per ml. The membranes were solubilized in Triton X-100, and a partially purified LDL receptor preparation was obtained by DEAE-cellulose chromatography (19). Typically, 20–50 g of bovine liver or brain (medulla and pons) yielded per gram of tissue 6.5–8.0 mg of solubilized brain membrane protein or 30–76 mg of solubilized liver membrane protein. A total of 300 mg of protein was loaded onto a 3-ml DEAE-cellulose column and eluted with a gradient of 0–200 mM NaCl. Following DEAE-cellulose chromatography, column fractions were fractionated by $NaDodSO_4$/7% polyacrylamide gels under nonreducing conditions and transferred to nitrocellulose filters (20). For immunoblotting, the filters were incubated for 1 hr at room temperature with 10 μg of mouse monoclonal antibody per ml directed against either the LDL receptor (IgG-15C8) (21) or an irrelevant antigen (IgG-2001) (20), washed, and then probed with alkaline phosphatase-conjugated goat anti-mouse IgG (Bio-Rad) and color reagents according to the manufacturer's instructions. For ligand blotting (22), the filters were incubated for 1 hr at 37°C with 5 μg of protein per ml of ^{125}I-labeled rabbit β-migrating very low density lipoprotein (β-VLDL; $\approx 3 \times 10^5$ cpm/μg of protein), washed, and exposed to XAR-5 film as indicated in the figure legends.

RESULTS

Fig. 1 shows a blot hybridization performed with total RNA isolated from various regions of the CNS of mature rabbits. After agarose gel electrophoresis and transfer to a nylon membrane, the RNA was probed with a mixture of ^{32}P-labeled fragments of cDNA corresponding to the coding region of the rabbit LDL receptor gene. For comparative purposes the membrane was also hybridized with a ^{32}P-labeled probe derived from a cDNA for human β-actin. An LDL receptor mRNA of approximately 3.3 kilobases (kb) in length was found in all of the brain fractions, and it was indistinguishable in size from the LDL receptor mRNA of liver. Within the CNS, mRNA levels were highest in the medulla/pons and in the spinal cord. Lower levels of receptor mRNA were found in the cerebral cortex, thalamus, hypothalamus, midbrain, and cerebellum. The amount of LDL receptor mRNA in the spinal cord and medulla/pons was in the same range as that found in liver of the same animal. All of the brain regions contained similar amounts of β-actin mRNA. The amount of β-actin mRNA appeared to be lower in liver than in brain.

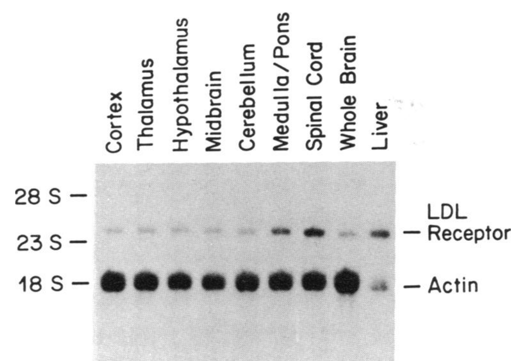

FIG. 1. Blot hybridization of LDL receptor mRNA from brain and liver of normal adult rabbits. Total RNA (10 μg) from each tissue was size-fractionated at room temperature on a 1.5% agarose gel for 16 hr at 30 V and transferred to a nylon membrane. The nylon membrane was then hybridized with a mixture of four uniformly ^{32}P-labeled rabbit cDNA probes and a human β-actin probe as described. The filters were washed briefly in 2× NaCl/Cit containing 1% $NaDodSO_4$ and then in 0.1× NaCl/Cit containing 1% $NaDodSO_4$ at 68°C for 90 min and then were exposed to XAR-5 film with an intensifying screen for 2 days at −70°C. The figure shows the data from one of three rabbits, all of which gave virtually identical results.

To confirm that the LDL receptor mRNA detected in the CNS was the product of the same gene that produced the liver mRNA, we performed an S1 nuclease analysis on mRNA obtained from normal and homozygous WHHL rabbits. The WHHL rabbit has a deletion of 12 base pairs in the coding region of the LDL receptor gene (12). When mRNA from the WHHL rabbit is hybridized to a uniformly labeled cDNA probe that encompasses this region, the probe is cleaved by the single-strand-specific nuclease because of the discontinuity between the wild-type and WHHL sequence (12). Fig. 2 shows that, in the brain and spinal cord of normal rabbits, the S1 nuclease analysis yielded a single protected fragment of 553 nucleotides, just as was obtained in the liver and adrenal from the same animals. On the other hand, the brain and spinal cord samples from the WHHL rabbit yielded two fragments of 292 and 249 nucleotides, which was identical to the result obtained with mRNA from the liver and adrenal gland of the same animals.

To study the changes in levels of mRNA for the LDL receptor during brain development, we obtained the brains and livers from developing rabbits at 3, 9, and 30 days after birth and from adult rabbits older than 5 months. Various amounts of total RNA were spotted on nitrocellulose filters by using a "slot-blot" apparatus, and the filters were incubated with the mixed ^{32}P-labeled LDL receptor cDNA probes (Fig. 3). The amount of receptor mRNA was remarkably constant from animal to animal and showed no significant variation from 3 days to adulthood. In the livers of the same animals, the amounts of LDL receptor mRNA were relatively low within month 1 of life, but they increased markedly by adulthood.

In the same experiment we also measured the levels of mRNA for HMG-CoA synthase, a regulated enzyme in the cholesterol biosynthetic pathway (Fig. 4). The mRNA for this enzyme has been shown to be regulated coordinately with HMG-CoA reductase and other enzymes of cholesterol

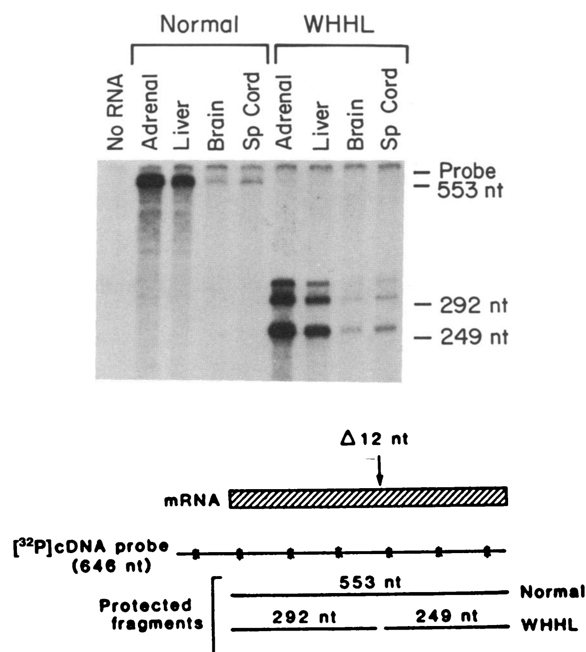

FIG. 2. S1 nuclease analysis of LDL receptor mRNA from adult normal and WHHL rabbits. LDL receptor mRNAs were analyzed exactly as described (12). Briefly, a uniformly ^{32}P-labeled probe corresponding to nucleotides 78–629 of the rabbit LDL receptor cDNA plus 93 nucleotides of cloning and phage M13 polylinker sequences was hybridized to 20 μg of total RNA isolated from the indicated tissues and then was digested with S1 nuclease. Protected fragments were visualized by autoradiography after fractionation on denaturing polyacrylamide gels. Lengths of the protected fragments were estimated relative to standards from *Hae* III-digested bacteriophage ϕX174 DNA. The slightly larger band above the 292-nucleotide fragment derived from the WHHL allele is a consequence of incomplete S1 digestion of poly(G) tails (generated in the cloning procedure) present at the 5′ end of the probe. The design of the experiments and the expected results for the normal and WHHL mRNAs are indicated in the lower portion of the figure. The gel was exposed to XAR-5 film for 2 days at −70°C with an intensifying screen. nt, Nucleotides.

biosynthesis in animal tissues (13). Moreover, relatively high levels of HMG-CoA synthase mRNA have been observed previously in hamster brain (22). Fig. 4 shows that the amount of HMG-CoA synthase mRNA in brain was highest in month 1 of life, but declined in adulthood. The amount of HMG-CoA synthase mRNA in the liver was more variable, and it showed no consistent change with age.

To quantify these data, the autoradiograms of Figs. 3 and 4 were subjected to scanning densitometry. We also performed scanning densitometry on autoradiograms from another experiment with three animals from each age group that were studied in a fashion identical to that shown in Figs. 3 and 4. The results of these two experiments are shown in Table 1. In both experiments the amount of LDL receptor mRNA in whole brain was essentially constant throughout development, whereas the amount of HMG-CoA synthase mRNA declined by 50–70% after age 30 days. In both experiments the amount of LDL receptor mRNA in liver was low during month 1 and increased by at least 5-fold during adulthood.

The low levels of mRNA for the LDL receptor in rabbit brain suggested that low levels of LDL receptor protein would be present. Several attempts to demonstrate the presence of LDL receptors in rabbit brain and spinal cord were unsuccessful because of the lack of a high-affinity antibody that recognizes the rabbit LDL receptor and the large amounts of myelin that limited recovery of membrane proteins. To circumvent these problems, we turned to the

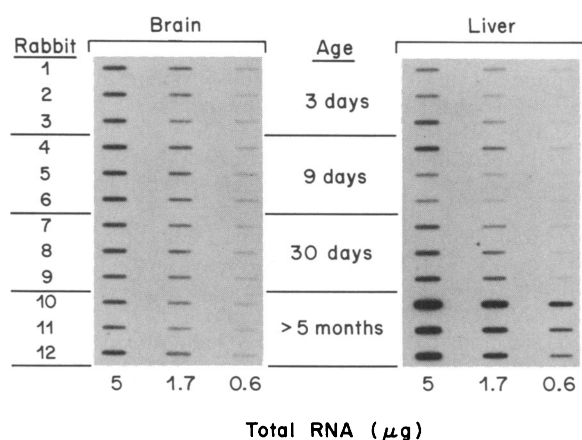

FIG. 3. Slot-blot hybridization of LDL receptor mRNA from normal rabbits at different ages. Total RNA was analyzed as described. Each row represents mRNA from the liver or brain of one rabbit. After hybridization, the nitrocellulose filters were washed in two changes of 2× NaCl/Cit containing 1% $NaDodSO_4$ at 23°C and then in 0.1× NaCl/Cit containing 1% $NaDodSO_4$ at 68°C for 90 min. The hybridization signal was quantified by scanning densitometry after autoradiography for 2 days at −70°C using an intensifying screen. The figure shows the data from one of two experiments, both of which gave similar results. The numerical values derived from densitometry from both experiments are presented in Table 1.

bovine brain. Homogenates from bovine medulla/pons were prepared, a microsomal membrane fraction was isolated, the membrane proteins were extracted with Triton X-100, and the extract was passed over a DEAE-cellulose column and eluted with a salt gradient. Fractions from the column were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated either with a monoclonal antibody that recognizes the bovine LDL receptor followed by addition of an ^{125}I-labeled second antibody or with ^{125}I-labeled β-VLDL, a ligand for the LDL receptor. We also performed the same experiments with liver extracts.

The bovine liver showed a peak of LDL receptor immunoreactivity (Fig. 5*A*) and ^{125}I-labeled β-VLDL binding activity (Fig. 5*B*) that was maximal in fraction 7 from the DEAE-cellulose column. When the extract from the medul-

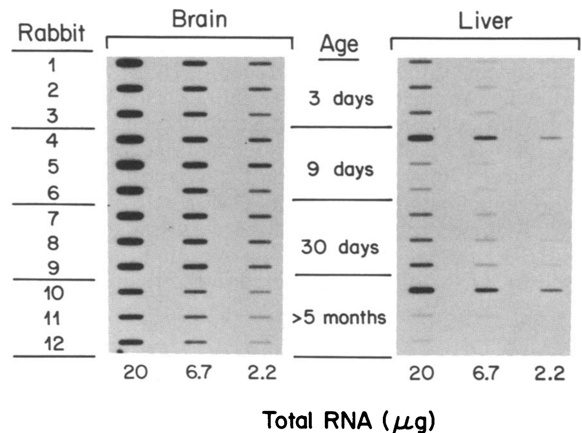

FIG. 4. Slot-blot hybridization of HMG-CoA synthase mRNA from normal rabbits at different ages. Aliquots of total RNA from the same samples presented in Fig. 3 were applied to nitrocellulose filters and hybridized with a ^{32}P-labeled HMG-CoA synthase probe as described. After hybridization, the filters were washed in two changes of 2× NaCl/Cit containing 1% $NaDodSO_4$ at 23°C and then in 0.1× NaCl/Cit containing 1% $NaDodSO_4$ at 53°C for 90 min. The figure shows the data from one of two experiments, both of which gave similar results. The numerical values derived from densitometry from both experiments are presented in Table 1.

Table 1. Quantification of LDL receptor and HMG-CoA synthase mRNA levels in whole brain and liver of rabbit at different ages

Exp.	mRNA	Tissue	Relative intensity at rabbit age			
			3 days	9 days	30 days	>5 months
A	LDL receptor	Brain	1.3	1.1	1.4	1.0
		Liver	1.1	1.0	1.8	5.3
	HMG-CoA synthase	Brain	2.5	2.6	2.2	1.0
		Liver	0.1	0.1	0.1	0.2
B	LDL receptor	Brain	0.9	1.2	0.8	1.0
		Liver	0.6	0.6	1.4	4.8
	HMG-CoA synthase	Brain	1.5	2.8	2.0	1.0
		Liver	0.2	0.4	0.2	0.3

Scanning densitometry was performed on autoradiograms of RNA slot blots probed with either ^{32}P-labeled LDL receptor or HMG-CoA synthase cDNA probes as shown in Figs. 3 and 4. Each value represents the mean intensity derived from three animals at the age indicated. Values are normalized to a mean intensity of 1.0 for the signal derived from the adult rabbit brain (>5 months) for each experiment and type of mRNA examined.

la/pons was chromatographed, a peak of receptor activity was seen in fractions 7 and 8 that bound both the monoclonal antibody (Fig. 5*C*) and β-VLDL (Fig. 5*D*). The molecular weight was the same as that of the receptor in the liver extracts. Fractions 7–9 contained a protein of ≈100 kDa that bound β-VLDL (Fig. 5*D*) but not the monoclonal antibody (Fig. 5*C*). The peak activity of this protein occurred in fraction 9 (Fig. 5*D*). When each fraction was incubated with ^{125}I-labeled β-VLDL in the presence of EDTA, binding to the high molecular weight LDL receptor was prevented, but binding to the lower molecular weight protein was not inhibited (Fig. 5*D*, lane C). Thus, the 100-kDa protein was not

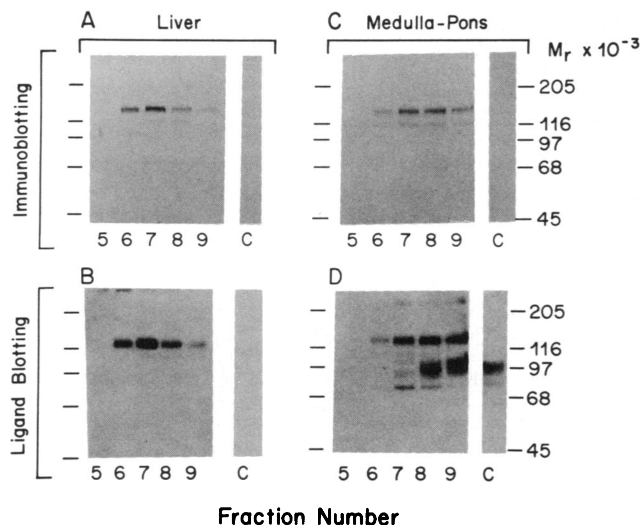

FIG. 5. Immunoblotting and ligand blotting of the LDL receptor from liver and brain of adult cows. Solubilized membranes from bovine liver (*A* and *B*) or the medulla/pons of bovine brain (*C* and *D*) were partially purified by DEAE-cellulose chromatography, subjected to $NaDodSO_4$ gel electrophoresis, and transferred to nitrocellulose filters. Immunoblots (*A* and *C*) were developed by using a mouse monoclonal anti-LDL receptor antibody (IgG-15C8) (lanes 5–9 in *A* and *C*) followed by alkaline phosphatase-conjugated goat anti-mouse IgG. Lanes C, for control, show the results obtained when fractions were incubated with a control monoclonal antibody (IgG-2001) in place of anti-receptor antibody. Only fraction 8 is shown. The lanes with the peak amounts of antibody binding each contained 50 μg of protein. Ligand blots (*B* and *D*) were performed on identical aliquots of the same column fractions as in *A* and *C* using ^{125}I-labeled β-VLDL as the ligand. Lanes C, for control, show the results obtained when fractions were incubated in the presence of 5 mM EDTA, which inhibits specific receptor–ligand interactions (19). Only fraction 8 is shown. The nitrocellulose filters in *B* and *D* were exposed to XAR-5 film for 6 hr at −70°C with an intensifying screen.

the LDL receptor. The nature of this ^{125}I-labeled β-VLDL binding protein was not further explored.

DISCUSSION

The current results show the presence of low but detectable levels of mRNA for the LDL receptor in various regions of the brain and in the spinal cord of immature and mature rabbits. The amounts of mRNA were highest in the medulla/pons and spinal cord, which are the most heavily myelinated of the regions that were studied. However, the levels of receptor mRNA did not correlate with the rate of myelin synthesis. The levels were the same during month 1 of life at which time myelination is most intense, as they were in mature animals when new myelin synthesis is minimal. Thus, it would seem that LDL receptors in the CNS are not present to provide cholesterol for the bulk synthesis of myelin during development.

The LDL receptor mRNA in the CNS was produced by the same gene as the one that produced the receptor mRNA in liver and adrenal gland, as indicated by its similar size and its hybridization with ^{32}P-labeled cDNA probes. Moreover, the mRNA produced in the brain and spinal cord of the WHHL rabbit showed evidence of the same small deletion that is known to be present in the LDL receptor gene of the WHHL rabbit. In experiments not shown, we could not detect any evidence for differential splicing of the LDL receptor mRNA in brain RNA preparations from normal rabbits.

Using a monoclonal antibody specific for the LDL receptor and using β-VLDL as a ligand for the receptor, we were able to demonstrate the presence of LDL receptors in bovine brain. It was difficult to estimate the number of such receptors relative to the liver because the recovery of membrane protein in the 100,000 × *g* pellet was only 1/10th as great in the brain as in the liver. Apparently, large amounts of myelin lipid impeded the performance of good cell fractionation with brain homogenates. The receptor in the bovine pons/medulla had the same molecular weight as the liver LDL receptor, and it adhered to DEAE-cellulose in a similar fashion.

The function of the LDL receptor in the CNS is not known. A ligand for the LDL receptor, apoE, is known to be secreted by brain astrocytes (10). If this secreted apoE binds to the LDL receptor, it might function to transfer cholesterol and/or phospholipid from astrocytes to other cells that express LDL receptors. Interpretation of such transport will depend on knowledge of the specific cells in the brain and spinal cord that produce LDL receptors. Because of the low level of receptor protein and mRNA, such demonstrations will be difficult unless the receptors are expressed at high levels in only a small fraction of cells.

Boyles *et al.* (23) have demonstrated that apoE is produced in distal segments of peripheral nerve during the demyelination reaction that follows crush injury. This apoE is believed to be secreted by the macrophages that invade the damaged nerve to ingest and degrade the myelin. The apoE, together with bound cholesterol, may then be taken up by growing neurons and by Schwann cells that could use the cholesterol for synthesis of new myelin (23). This uptake is likely to be mediated by LDL receptors. Indeed, LDL receptors have been demonstrated recently in cell bodies and growth cones of growing neurons derived from cultured mouse pheochromocytoma (PC12) cells (24). It is possible that LDL receptors might play a role in repairing injury in the brain and spinal cord. It will be important to know whether the number of LDL receptors and the secretion rate of apoE rises in response to inflammation, degeneration, or trauma in the brain or spinal cord.

The LDL receptor cannot be absolutely essential for brain development. Some humans with the homozygous form of familial hypercholesterolemia are totally unable to produce any LDL receptors as a result of a deletion that removes the 5′ end of the LDL receptor gene (25). These individuals do not have any apparent difficulty with CNS development or function. Whether they have some difficulty in repairing damaged nerves is unknown. In other instances in which LDL receptors are deficient, backup mechanisms come into play to supply cells, such as adrenal cells, with cholesterol. It is possible that, in the face of receptor deficiency, backup mechanisms in the CNS perform a function that is normally performed by LDL receptors.

An unexpected finding in the current studies was the 5-fold increase in LDL receptor mRNA in the livers of adult rabbits as compared with rabbits that were less than 1 month of age. The younger animals were studied during the suckling period and, thus, were consuming a relatively high fat diet, which is known to suppress LDL receptor mRNA levels in adult animals (P. Ma, J.L.G., and M.S.B., unpublished observations). However, other factors might be involved. All of the adult animals that were studied were male. Previous studies by Zilversmit and Hughes (26) showed that the plasma cholesterol level drops dramatically in male rabbits but not in female rabbits between 10 and 20 weeks of age. It is possible that some hormonal factor in male rabbits leads to a stimulation of LDL receptors, which in turn causes the LDL level to fall.

In previous studies with solution hybridization techniques, we estimated that the livers of adult male rabbits contain an average of about 40–80 copies of LDL receptor mRNA per cell (27). Although the concentration of mRNA is low, the liver produces a relatively high number of LDL receptors because of the large size of the organ. The levels of receptor mRNA in the medulla/pons and spinal cord appeared to be about the same as that in the liver (Fig. 1). If the expression of mRNA were confined to a small fraction of cell types in these neural tissues, then it is possible that receptor activity in a given cell type would be of quantitative importance in regulating cholesterol metabolism of that cell type.

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