Short-term effects of ACTH on the low-density lipoprotein receptor mRNA level in rat and hamster adrenals

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ABSTRACT

Low-density lipoprotein (LDL) receptor mRNA was found in both rat and hamster adrenals. Within 30 min after ACTH administration a significant increase in the levels of both LDL receptor and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) mRNAs was observed in rat adrenals; these levels remained increased for up to 240 min. The increase in the levels of LDL receptor and HMG-CoA reductase mRNAs produced by ACTH was reduced by co-administration of aminoglutethimide while, at the same time, the adrenal cholesterol content of rats treated with both aminoglutethimide and ACTH was significantly increased compared with that in groups treated with ACTH alone. Cycloheximide also induced increased levels of rat adrenal mRNAs for LDL receptor and HMG-CoA reductase, but this effect was not additive with that of ACTH. These results suggest that, in the rat, the short-term effect of ACTH on the levels of mRNAs for the LDL receptor and HMG-CoA reductase is similarly controlled and might be mediated through changes in the adrenal cholesterol content. In the hamster adrenal, however, no significant fluctuations were found in the level of LDL receptor mRNA, although a marked increase was found in the level of HMG-CoA reductase mRNA, 2 h after ACTH administration. This indicates that an important effect of ACTH on cholesterol metabolism in the hamster adrenal is at the level of HMG-CoA reductase. In the hamster, therefore, where the main source of cholesterol for the adrenal gland is de-novo synthesis, it seems that a complex mechanism is involved in the control of LDL receptor gene expression.

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INTRODUCTION

In steroid-metabolizing tissues, the precursor cholesterol originates from three sources: de-novo cholesterol synthesis, cholesterol uptake from plasma lipoproteins and utilization of endogenously stored cholesterol (Lehoux, 1979). Recent studies conducted in vitro on cell cultures from various tissues, including non-steroid-producing tissues, have clarified different aspects of the molecular control of gene expression of the low-density lipoprotein (LDL) receptor responsible for cholesterol uptake (Russell, Yamamoto, Schneider et al. 1983; Südhof, Russell, Brown & Goldstein, 1987; Dawson, Hofmann, van der Westhuyzen et al. 1988; Auwerx, Chait & Deeb, 1989; Molowa & Cimis, 1989; Smith, Osborne, Goldstein & Brown, 1990) and of the key regulatory enzyme for cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Gil, Osborne, Goldstein & Brown, 1988a; Gil, Smith, Goldstein et al. 1988b; Nakaniishi, Goldstein & Brown, 1988; Osborne, Gil, Goldstein & Brown, 1988; Metherall, Goldstein, Luskey & Brown, 1989; Molowa & Cimis, 1989; Rosser, Ashby, Ellis & Edwards, 1989). Sequence designated sterol regulatory elements (SRE-1) were found in the promoters of LDL receptor (Smith et al. 1990) and HMG-CoA reductase (Osborne et al. 1988) genes, which are inactivated by sterols. In the LDL receptor promoter, transcription is enhanced in the absence but inactivated in the presence of sterols (Smith et al. 1990), while in the HMG-CoA reductase promoter, SRE-1 represses transcription in the presence of sterols (Gil et al. 1988a,b; Osborne et al. 1988).

From the above in-vitro studies, it seems probable that HMG-CoA reductase and LDL receptors could be co-ordinately controlled. In-vivo studies on the

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source of cholesterol used for steroidogenesis, however, showed large differences between animal species and even between tissues of the same animal (Lloyd, 1972; Lehoux, 1979; Lehoux & Lefebvre, 1980; Spady & Dietschy, 1985), involving subtle control mechanisms at this level. For example, it was reported that the rate of cholesterol synthesis was 24-fold greater in the hamster than in the rat adrenal, whereas the rate of LDL-cholesterol uptake was much higher in the rat than in the hamster adrenal (Spady & Dietschy, 1985). In vivo, both hamster (Lehoux, Lefebvre, de Médicis et al. 1987) and rat (Balasubramaniam, Goldstein, Faust et al. 1977; Lehoux et al. 1987; Lehoux, Lefebvre, Bélisle & Bellabarba, 1989a, 1990) adrenal HMG-CoA reductase activity is under the control of adrenocorticotropic hormone (ACTH). LDL receptor-binding sites were also shown to be under the control of ACTH in the rat adrenal (Kovanen, Goldstein, Chappell & Brown, 1980). We took advantage of differences, between the rat and the hamster, in the source of cholesterol used in the adrenal to study the short-term effect of ACTH on the level of HMG-CoA reductase and LDL receptor mRNAs.

MATERIALS AND METHODS

Materials

\(^{[32P]}\text{dCTP (3000 Ci/mmol)}\) was purchased from Amersham Canada, Oakville, Ontario, Canada, and glyoxal from Anachemia Canada, Ville St Pierre, Quebec, Canada. Aminoglutethimide was a gift from Ciba Geigy, Summit, NJ, U.S.A., and ACTH (Duracon) was from Nordic Pharmaceuticals, Laval, Quebec, Canada. Cycloheximide was obtained from Sigma, St Louis, MO, U.S.A. All other chemicals were of analytical grade.

Animals

Male Long–Evans rats (250±25 g) were obtained at 2 months of age from our local breeding colony. Male Syrian hamsters (100±10 g) were purchased from Charles River, Canada Inc., St Constant, Quebec, Canada, and kept in an isolated room with a controlled light/dark cycle (lights on from 06.00 to 18.00 h) for 3 weeks before experimentation. Purina rat chow and tap water were available ad libitum. Animals were always killed at the same time of day.

Treatments

Groups of animals were injected S.C. with 1 IU ACTH/100 g body weight and killed after various times. When used, 2 mg aminoglutethimide/100 g body weight were co-administered with ACTH. In another series of experiments, aminoglutethimide was co-injected with cycloheximide (1 mg/100 g body weight). Controls were injected with 0·15 M NaCl.

Isolation of mRNA

The liver and adrenals were homogenized in 15 volumes of a 7 M guanidine–HCl solution containing 20 mM iodoacetic acid, 1% (w/v) lauroyl sarcosine and 1 mM EDTA (pH of -20°C). RNA was isolated as previously described (Lehoux et al. 1990) and the RNA content estimated by spectrophotometry at 260 and 280 nm.

RNA electrophoresis and blotting

Total RNA was denatured with glyoxal (Thomas, 1983), electrophoresed in 1% (w/v) agarose in 0·01 M phosphate buffer (pH 6·5) and transferred to GeneScreenPlus (Dupont Canada Inc., Mississauga, Ontario, Canada). The membrane was then prehybridized for 16 h at 43°C and hybridized for 24 h at 43°C (Liscum, Luskey, Chin et al. 1983). Blots were probed (1) with a \(^{32P}\)-labelled reductase cDNA prepared from pRed-10 (Chin, Luskey, Faust et al. 1982) and kindly provided by Dr M.S. Brown, University of Texas Health Science Center at Dallas, TX, U.S.A., and (2) with a \(^{32P}\)-labelled human LDL receptor cDNA obtained from the American Type Culture Collection, Rockville, MD, U.S.A. The cDNA probes were labelled to a specific activity of 1–2 \times 10^9\ c.p.m./\mu g and used for hybridization at a concentration of 2 ng/ml. In some experiments, mRNA levels were measured by a cytoplasmic dot hybridization technique (White & Bancroft, 1982). The blots were also analysed with a \(^{32P}\)-labelled ribosomal probe for the standardization of molecular masses of quantities of RNAs (Lehoux et al. 1990). \(\beta\)-actin mRNA was analyzed with a human \(^{32P}\)-labelled \(\beta\)-actin cDNA probe.

Autoradiograms were obtained by exposing the blots to Kodak X-Omat RP films with Cronex Lightning plus enhancing screens (Dupont Canada Inc.). Decreasing concentrations of total RNA were analysed for each group, and blots were exposed for different times to ensure linearity of measurement. The intensity of bands on the films was determined using an LKB 2222-020 Ultrascan XL laser densitometer (Pharmacia Canada Ltd, Dorval, Quebec, Canada).

Cholesterol determination

Adrenal cholesterol levels were determined using o-phthalaldehyde (Rudel & Morris, 1973). Correc-
tions for losses during manipulation were made using \(^{3}H\)cholesterol and \(^{14}C\)cholesteryl oleate.

**Corticosteroid determination**

Plasma corticosteroids were measured as described previously (Legros & Lehoux, 1983).

**RESULTS**

**Presence of LDL receptor mRNA in rat and hamster**

Figure 1 demonstrates the presence of LDL receptor mRNA in both rat and hamster adrenals. Northern analyses detected these mRNAs in the 5.3 kb area, at the same level as that of liver mRNAs.

**Short-term effect of ACTH on LDL receptor and HMG-CoA reductase mRNAs**

Figure 2 shows the effect of ACTH on the levels of rat adrenal LDL receptor mRNA and plasma corticosteroid. The levels of both rapidly increased in parallel to reach a plateau at 30 min after ACTH administration and remained increased up to 240 min (Fig. 2b). Figure 2 also shows that the level of rat adrenal HMG-CoA reductase mRNA was also significantly increased 30 min after ACTH administration, and remained raised up to 240 min, while the level of \(\beta\)-actin mRNA remained unchanged during the experiment.

A different situation was found with the hamster adrenal, as the level of LDL receptor mRNA did not change significantly after ACTH administration (Fig. 3b), although the plasma corticosteroid level was significantly increased 30 min after treatment (Fig. 3a). In the same series of experiments the level of HMG-CoA reductase mRNA was significantly increased 2 h after ACTH treatment, and decreased thereafter at 3 and 4 h. (Fig. 2b)

Figure 4 shows that, under normal conditions, the level of HMG-CoA reductase mRNA is much higher in the hamster than in the rat adrenal. Identical results were obtained using a \(^{32}\)P-labelled cDNA probe originating from either a rat or hamster library.

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**FIGURE 1.** Northern analysis of low-density lipoprotein (LDL) receptor mRNA in the adrenal and liver of the rat and hamster. Total RNA (20 µg/lane) was electrophoresed on a 1.0% agarose gel, blotted and hybridized to \(^{32}\)P-labelled human LDL receptor cDNA.

**FIGURE 2.** Effects of administration of ACTH on the levels of (a) plasma corticosteroid (\(\bullet\)) and (b) adrenal low-density lipoprotein (LDL) receptor (\(\triangle\)), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (\(\diamondsuit\)) and \(\beta\)-actin (\(\square\)) mRNAs in rats. Groups of rats were injected with 1 IU ACTH/100 g body weight and killed at the times indicated. Total RNA was analysed by the cydotol hybridization technique using \(^{32}\)P-labelled LDL receptor, \(^{32}\)P-labelled HMG-CoA reductase or \(^{32}\)P-labelled \(\beta\)-actin cDNA. Values are means ± S.E.M., \(n=3\). *\(P<0.05\), **\(P<0.02\), ***\(P<0.01\), ****\(P<0.001\) compared with control groups (analysis of variance).
Effect of ACTH or ACTH in combination with aminoglutethimide on the levels of rat adrenal LDL receptor mRNA

Since we found no significant short-term effect of ACTH on the LDL receptor mRNA level in the hamster adrenal, the following series of experiments was performed on the rat adrenal only. In these experiments, animals were killed 180 min after drug administration. Figure 5 and Table 1 show that aminoglutethimide, a drug inhibiting the conversion of cholesterol to pregnenolone, lowered the enhancing effect of ACTH on both the LDL receptor and HMG-CoA reductase mRNA contents. Table 1 also shows that ACTH caused a decrease in the adrenal esterified cholesterol content and that aminoglutethimide prevented this decrease. In addition, the free cholesterol content of adrenals was significantly increased following the co-administration of aminoglutethimide with ACTH.

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TABLE 1. Effect of ACTH (1 IU/100 g body weight) or ACTH (1 IU/100 g) in combination with aminogluthethimide (AG; 2 mg/100 g) on the levels of rat adrenal cholesterol, low-density lipoprotein receptor (LDL-R) mRNA and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA, 3 h after treatment. Values are means ± s.e.m., n = 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LDL-R mRNA (relative level)</th>
<th>HMG-CoA reductase (relative level)</th>
<th>Free cholesterol (relative level)</th>
<th>Esterified cholesterol (relative level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00±0.13</td>
<td>1.00±0.05</td>
<td>1.00±0.04</td>
<td>1.00±0.06</td>
</tr>
<tr>
<td>ACTH</td>
<td>1.95±0.07**</td>
<td>2.14±0.27*</td>
<td>0.90±0.02</td>
<td>0.68±0.04*</td>
</tr>
<tr>
<td>ACTH + AG</td>
<td>1.46±0.07</td>
<td>1.27±0.14</td>
<td>1.29±0.06*</td>
<td>1.01±0.05</td>
</tr>
</tbody>
</table>

*P <0.02, **P <0.01 compared with control (analysis of variance).

Control, free cholesterol content: 14.2±0.6 μg/mg protein; esterified cholesterol: 74.7±4.6 μg/mg protein.
Control rats were injected with 0.15 m NaCl.

Effect of cycloheximide and ACTH on the level of rat adrenal LDL receptor mRNA

Figure 6 shows the results of a time study on the effect of cycloheximide, a protein-synthesis inhibitor which also blocks corticosteroidogenesis, on the levels of LDL receptor and HMG-CoA reductase mRNAs. The level of both mRNAs was significantly increased 2 h after cycloheximide administration and remained increased up to 4 h. Figure 7 shows that cycloheximide was as potent as ACTH in inducing an increase in the level of rat adrenal LDL receptor mRNA. The action of cycloheximide was not additive to that of ACTH. Figure 7 also shows that cycloheximide significantly increased the adrenal level of HMG-CoA reductase mRNA, and that this effect was also not additive to that of ACTH.

**FIGURE 6.** Effect of cycloheximide on the levels of (a) low-density lipoprotein (LDL) receptor and (b) 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA in the rat adrenal. Groups of rats were injected with 0.15 m NaCl or 1 mg cycloheximide/100 g body weight and killed at the times indicated. RNA was analysed by the cytodot hybridization technique. Cytosol was blotted and hybridized to 32P-labelled LDL receptor or 32P-labelled HMG-CoA reductase cDNA. Values are means ± s.e.m., n = 3. *P <0.05, **P <0.01, ****P <0.001 compared with control groups (analysis of variance).

**FIGURE 7.** Effect of ACTH (A) or ACTH plus cycloheximide (CX) on the levels of (a) low-density lipoprotein (LDL) receptor and (b) 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNAs in the rat adrenal. Groups of rats were injected with 0.15 m NaCl (control; C), 1 IU ACTH/100 g body weight, 1 mg cycloheximide/100 g body weight or ACTH plus cycloheximide and killed 180 min later. RNA was analysed by the cytodot hybridization technique. Cytosol was blotted and hybridized to 32P-labelled LDL receptor or 32P-labelled HMG-CoA reductase cDNA. Values are means ± s.e.m., n = 3. *P <0.05, **P <0.02, ****P <0.001, *****P <0.001 compared with control groups (analysis of variance).

**DISCUSSION**

Two animal models were studied in the present experiments: the rat, which uses mainly circulating plasma lipoprotein cholesterol for adrenal steroidogenesis, and the hamster, which synthesizes de novo the majority of the adrenal cholesterol used for its steroidogenesis. Northern analysis revealed that...
mRNAs from the hamster and rat adrenal and liver hybridized at the same area with the human cDNA probe for the LDL receptor. The size of the hamster and rat adrenal LDL receptor mRNA was estimated at about 5-3 kb, which is in agreement with the size reported for LDL receptor mRNA of the bovine adrenal, of human culture epidermal carcinoma A-431 cells (Russell et al. 1983) and of human granulosa cells (Takagi & Strauss, 1989).

We also compared the short-term effects of ACTH on the levels of adrenal LDL receptor and HMG-CoA reductase mRNAs between the rat and hamster. Our results showed a short-term enhancement of LDL receptor mRNA level in the rat adrenal. This effect was rapid and sustained for 240 min after administration of ACTH, and correlated well with previous findings indicating a rapid increase produced by ACTH on the number of membrane sites specifically binding $^{125}$I-labelled LDL (Kovanen et al. 1980). A short-term enhancement of the level of HMG-CoA reductase mRNA by ACTH was also observed in the rat adrenal. Indeed, a significant twofold increase was found 30 min after ACTH administration. This is in agreement with previous reports that ACTH provoked significant increases in rat adrenal HMG-CoA reductase activity (Balasubramaniam et al. 1977; Lehoux et al. 1987) and reductase protein (Lehoux et al. 1987) 3 h after ACTH administration. In contrast to rats, in hamster adrenals there were no significant fluctuations in the level of LDL receptor mRNA up to 300 min after the administration of ACTH, but in the same preparations significant increases in the level of HMG-CoA reductase mRNA were found 2, 3 and 4 h after ACTH administration. There are no other reports on the level of LDL receptor mRNA in the hamster adrenal that can be used to compare with those of the present study. The above results indicate that although the gene for the LDL receptor is expressed in adrenals of the hamster, insofar as the level of LDL receptor is concerned, this gland does not respond to ACTH in the same manner as the rat adrenal. Our results on the effect of ACTH on the level of HMG-CoA reductase mRNA in the hamster adrenal are in agreement with previous findings (Lehoux et al. 1987; Lehoux, Lefebvre, de Médicis et al. 1989b). The basal level of HMG-CoA reductase mRNA was much lower in rat than in hamster adrenals (Fig. 4), and therefore a twofold increase in these levels is surely quantitatively far less important in the rat than in the hamster adrenal. This is in agreement with the fact that HMG-CoA reductase activity is normally 18-fold higher in the hamster than in the rat adrenal (Lehoux et al. 1989a). The rat adrenal, however, responds more quickly than the hamster adrenal (30 min for rat compared with 120 min for hamster) to ACTH stimulation with regard to the levels of HMG-CoA reductase mRNA.

In the rat, aminoglutethimide, an inhibitor of the catabolism of cholesterol to pregnenolone, decreased the enhancing effect of ACTH on the level of adrenal LDL receptor mRNA. This drug also prevented the decrease in the cholesterol content of adrenals observed after administration of ACTH alone. These results indicate that, in the rat, the effect of ACTH on the level of LDL receptor mRNA might be associated with changes in the adrenal cholesterol content. These results are in agreement with other reports indicating that LDL receptor gene expression is inhibited in the presence of sterols (Dawson et al. 1988; Metherall et al. 1989; Smith et al. 1990). Similar inhibition of the mRNA level of the enzyme HMG-CoA reductase was seen following administration in vivo of ACTH together with aminoglutethimide to rats. These results are also in agreement with previous studies which demonstrated that HMG-CoA reductase activity and the level of HMG-CoA reductase mRNA varied inversely with the cholesterol content of adrenals (Balasubramaniam et al. 1977; Lehoux et al. 1987, 1990). Indeed, reductase activity and the reductase mRNA content are increased when the adrenal cholesterol content is lowered, and they are decreased when it is elevated. In addition, in-vitro studies indicate that the expression of the LDL receptor (Smith et al. 1990) and HMG-CoA reductase genes (Metherall et al. 1989) is at least partly controlled at the promoter level by sterol regulatory elements.

To gain an insight into the above-mentioned control mechanisms, we studied the activity of the translation inhibitor cycloheximide in the rat adrenal. This inhibitor of protein synthesis by itself increased both LDL receptor mRNA and HMG-CoA reductase mRNA levels. These results are in agreement with previous reports on the effect of cycloheximide on LDL receptor mRNA (Leitersdorf, Banai, Friedman & Kaempfer, 1989; Mazzone, Basheeruddin & Duncan, 1989) and on HMG-CoA reductase mRNA (Lehoux et al. 1989b).

We found no additive effect on the levels of these mRNAs when ACTH was co-administered with cycloheximide. The cholesterol content of rat adrenals was not decreased following cycloheximide administration to rats. In contrast, the free cholesterol content was significantly increased (control: $1.00 \pm 0.04$; cycloheximide: $2.64 \pm 0.12$, $P<0.001$; cycloheximide plus ACTH: $2.73 \pm 0.05$, $P<0.001$; means $\pm$ s.e.m.). This increased adrenal cholesterol content was presumably due to inhibition of sterol biosynthesis leading to an accumulation of non-metabolized substrate. These results indicate that a
high content of adrenal cholesterol is of itself insufficient to block the increase in LDL receptor and HMG-CoA reductase mRNAs, and are in agreement with the concept that the accumulation of LDL receptor mRNA is down-regulated by a labile protein which prevents expression of the gene (Auwerx et al. 1989). Leitersdorf et al. (1989) reported that in glioblastoma-astrocytoma cells, the LDL receptor mRNA remains highly unstable in the presence of cycloheximide, which does not support the concept of RNA stabilization as a cause of the increase they found in their system, as has been suggested for other genes (Lindsten, June, Ledbetter et al. 1989). Our results, however, do not allow us to confirm this assertion.

As mentioned above, there is a profound difference between rat and hamster adrenals concerning the origin of cholesterol used for corticosteroidogenesis. In the hamster, de novo intra-glandular synthesis is the major source of cholesterol (Lloyd, 1972; Lehoux & Lefebvre, 1980). As reported by Spady & Dietschy (1985), however a small fraction of the hamster adrenal cholesterol comes from receptor-dependent uptake of LDL (6 μg/h per g). In the present study we found that during short-term experiments this small LDL uptake by the hamster adrenal does not appear to be regulated by ACTH. In contrast, the rat adrenal gland synthesizes de novo much less cholesterol (Lloyd, 1972; Lehoux & Lefebvre, 1980; Spady & Dietschy, 1985) than the hamster (11- to 24-fold less) (Lehoux & Lefebvre, 1980; Spady & Dietschy, 1985), but its acquisition of cholesterol by receptor-dependent LDL uptake was found to be threefold greater than that of the hamster (18 μg/h per g) (Spady & Dietschy, 1985). Although greater than in the hamster adrenal, this LDL uptake is much less than that of high-density lipoproteins (4500 μg cholesterol/h per g) (Andersen & Dietschy, 1981), which are the major source of cholesterol for the rat adrenal. However, the cholesterol acquisition by de novo intra-glandular cholesterol synthesis and LDL uptake can be extremely important for the fine regulation of cholesterol homeostasis in the rat adrenal. Indeed, our results showed that the level of LDL receptor and HMG-CoA reductase mRNAs increases rapidly when challenged by ACTH, and that this effect can be prevented by aminoglutethimide. This means that there are rapid positive as well as negative responses at these levels that could contribute to fine adjustments of the adrenal cholesterol content needed for corticosteroidogenesis.

In conclusion, the two in vivo models used for the present experiments allowed the demonstration of the existence of subtle differences in the control of the level of LDL receptor mRNA by ACTH. These results point to the presence in vivo of possible unidentified factors that could differentially affect the expression of the LDL receptor gene in the adrenal glands of different species. These models could thus be used to study further the fine mechanisms involved in the control of adrenal steroidogenesis under physiological conditions.

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