Chapter VIII

EFFECT OF HORMONAL FACTORS ON THE SYNTHESIS AND SECRETION OF APOLIPOPROTEIN B BY HEPATOCYTES IN CULTURE

Hormones are known to influence the synthesis and secretion of VLDL. The hormones investigated in this respect include insulin, glucagon, epinephrine, glucocorticoids and α-estradiol. The available information on the effect of some of these hormonal factors has been discussed in the introduction. It is evident that very few detailed reports seem to be available on the effect of hormonal factors on the synthesis and secretion of apoB by hepatocytes in culture. Many of the reports available relate to results obtained using perfused liver preparations. In many cases the available reports themselves are not in agreement. In view of these, it was considered necessary to investigate the effect of hormonal factors, on the synthesis and secretion of apoB by hepatocytes in culture. The effect of in vitro addition of thyroxine, dexamethasone, cortisone, epinephrine, cyclic AMP, insulin, glucagon and β-estradiol on the synthesis and secretion of apoB has been studied. The effect of in vivo suppression of thyroid and of administration of thyroxine on the synthesis and secretion of apoB by hepatocytes isolated from the animals, has also been studied. The results are discussed in this chapter.
Materials and methods

L-thyroxine, dexamethasone, insulin, glucagon, epinephrine, dibutyryl cyclic AMP, corticosterone and β-estradiol were purchased from Sigma Chemicals (USA). Procedures used for preparation of hepatocytes from normal rat liver and their culture in vitro, metabolic labelling with (³H)-leucine and immunoprecipitation of apoB and all other procedures used are the same as described in chapter II.

Section I

The effect of in vitro addition of corticosterone and dexamethasone on the synthesis and secretion of apoB by rat hepatocytes in culture

As discussed in the introduction, corticosteroids have been reported to influence the synthesis and secretion of VLDL by hepatocytes. Addition of dexamethasone to culture medium of hepatocytes has been found to result in a stimulation of VLDL-TG and cholesterol secretion. Martin-Sanz et al recently reported that dexamethasone also increased the incorporation of (³H)-leucine into apoB of VLDL secreted by rat hepatocytes in culture. This report appeared while our investigations on the effect
of dexamethasone on the synthesis and secretion of apoB by rat hepatocytes in culture were just completed.

It has been reported that the addition of glucocorticoids in vitro to the culture medium did not reverse the inhibitory effect of insulin on VLDL-TG and apoB secretion in normal hepatocytes\textsuperscript{149,280}. By contrast, cortisol reversed the inhibitory effect of insulin on apoB secretion in hepatocytes from diabetic rats\textsuperscript{280}. Apart from these reports there does not seem to be any other report on the effect of addition of corticosterone on the synthesis and secretion of apoB by rat hepatocytes. The effect of in vitro addition of dexamethasone and corticosterone on the synthesis and secretion of apoB by rat hepatocytes in culture has been studied and the results are discussed in this section.

Results

1. Effect of in vitro addition of dexamethasone on the synthesis and secretion of apoB: The results are given in table (28) and 't' values are given in table (28a).

In vitro addition of dexamethasone has been found to increase the secretion of apoB into the medium when compared to the control cells. Cell associated apoB also showed a significant increase indicating increased synthesis of apoB.
Table. 28

Incorporation of \(^3\text{H}\)-leucine into apoB - Effect of dexamethasone and cortisone

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Media ([\text{CPM/mg cell protein}])</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1038±78</td>
<td>828±85</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1941±148</td>
<td>1314±120</td>
</tr>
<tr>
<td>Cortisone</td>
<td>1643±134</td>
<td>1469±108</td>
</tr>
</tbody>
</table>

Hepatocytes were incubated with \(^3\text{H}\)-leucine (20\(\mu\)Ci) in presence of dexamethasone \((10^{-6} \text{M})\) and corticosterone \((10^{-6} \text{M})\) for 12 hours. The radioactivity associated with secreted and cell associated apoB was measured. The results given are the mean of four different experiments ± SD. On comparison with control, \(p < 0.01\) in all cases.

Table. 28a

'\(t\)' values

<table>
<thead>
<tr>
<th>'(t)' between control &amp; hormone</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>10.79</td>
<td>6.61</td>
</tr>
<tr>
<td>Cortisone</td>
<td>7.80</td>
<td>9.33</td>
</tr>
</tbody>
</table>
2. Effect of corticosterone on the synthesis and secretion of apoB:

Results are given in table (28) and 't' values are given in table (28a). There was significant increase in the secretion of apoB into the medium on the addition of corticosterone when compared to the control. As in the case of dexamethasone cell associated apoB also showed a significant increase in the presence of corticosterone, indicating an increased production of apoB by hepatocytes in presence of corticosterone.

Section II

Effect of in vitro addition of β-estradiol on the synthesis and secretion of apoB

Sex steroids are known to be important regulators of apolipoprotein synthesis and secretion in a wide variety of species. In human, epidemiological studies have established strong correlation between sex steroid levels, circulating apolipoprotein profiles and atheroslerotic risk$^{281,282}$. The decrease in estrogen that occurs following menopause is reported to be accompanied by an elevation in the ratio of VLDL/HDL and a relatively abrupt increase in atheroslerotic risk$^{283}$. It is suggested that in women, normal physiological concentration of estrogen confers some protection against the development of atherosclerosis$^{283}$. It is clear from many other
studies that higher concentration of the hormone such as those found during pregnancy and during therapy with some type of oral contraceptives can induce marked increase in VLDL/HDL ratio and result in hypertriglyceridemia\textsuperscript{284,285}. At truly pharmacological levels, the hormone can elicit a pronounced hypolipidemic response and it has been used successfully to lower serum lipoprotein levels in individuals suffering from a familial hyperlipoproteinemia, dysbetalipoproteinemia\textsuperscript{286}. Studies on animal models, rat and rabbit indicate that the hypolipidemic responses to pharmacological doses of estrogen is possibly the result of a hormonally induced increase in the concentration of apoB/E receptors that are involved in the clearance of chylomicron and VLDL-remnants\textsuperscript{283}.

Estrogen dependence of avian apolipoprotein synthesis has been demonstrated\textsuperscript{174-176}. Similarly estrogen dependent modulation of lipoproteins in human hepatoma cells (HepG2 cells) has also been reported\textsuperscript{283}. However no report seems to be available on the effect of estrogen on the synthesis and secretion of apoB by hepatocytes. In view of this, the effect of $\beta$-estradiol on the synthesis and secretion of apoB by rat hepatocytes in culture has been studied.

Results

1. Effect of in vitro addition of $\beta$-estradiol on the synthesis and secretion of apoB: The results are given in table (29) and 't' values are given in table (29a).
Table. 29

Incorporation of (\(^3\)H)-leucine into apoB - Effect of \(\beta\)-estradiol

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[CPM/mg cell protein]</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1038±78</td>
<td>828±85</td>
</tr>
<tr>
<td>(\beta)-Estradiol</td>
<td>1820±82</td>
<td>1140±49</td>
</tr>
</tbody>
</table>

Hepatocytes were incubated with (\(^3\)H)-leucine (20\(\mu\)Ci) in presence of \(\beta\)-estradiol (10\(^{-6}\) M) for 12 hours. The radioactivity associated with the apoB secreted into the medium as well as that associated with the cell layer was measured. The results given are the mean of four different experiments ± SD. On comparison with control, \( p < 0.01 \)

Table. 29a

'\(t\)' values

<table>
<thead>
<tr>
<th>'(t)' between control &amp; hormone</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-Estradiol</td>
<td>13.82</td>
<td>6.36</td>
</tr>
</tbody>
</table>
There was significant increase in the secretion of apoB into the medium in the presence of $10^{-6}$ M concentration of $\beta$-estradiol when compared to control. Similarly the cell associated apoB also showed an increase at $10^{-6}$ M.

Section III

Effect of thyroxine on the synthesis and secretion of apoB by rat hepatocytes

The available reports on the effect of thyroid hormone on the synthesis and secretion of VLDL have been discussed in the introduction. It has also been mentioned that some of the observations made in this respect are contradictory. Although there appears to be general agreement that in vitro liver preparations from hyperthyroid rats secreted less VLDL-TG than normal, hepatic VLDL output in vivo is unchanged or increased. These apparent contradictory observations show that the action of thyroid hormone is complex. Most of the results reported relate to the synthesis and secretion of apoB from hypothyroid or hyperthyroid rats. There are very few reports on the addition of the hormone in vitro on the synthesis and secretion of apoB by hepatocytes. This aspect has now been studied. In another experiment rats were rendered hypothyroid by administration of thiouracil and the effect of hypothyroidism on the synthesis and secretion of apoB by the
hepatocytes isolated from them were studied. The effect of administration of thyroxine along with thiouracil on the synthesis and secretion of apoB was also investigated. The results are discussed in this section.

Thyroid suppression: Male albino rats (Sprague-Dawley strain, average weight 150g) were divided into three groups of 6 rats each. Group I - control rats, Group II-rats given thiouracil, Group III-rats given thiouracil + thyroxine. Thiouracil dissolved in normal saline, pH adjusted to 8.0 with dilute NaOH, (1mg/100g body weight) was administered daily by subcutaneous route for 14 days to rats of group II & III. 1µg of thyroxine per 100g body weight was administered to the rats of group-III in addition to thiouracil. The duration of the experiment was 14 days. The rats were caged individually in rooms maintained at 24±1°C with alternate exposure to light and dark for 12 hours each. Water was available ad libitum. At the end of this period, the animals, were deprived of food overnight and the liver was removed for isolation of hepatocytes as described in chapter II. The animals in all groups were fed normal laboratory diet throughout the experiments. Diet consumption was adjusted to be the same in the rats of various groups.

Results

1. Effect of addition of thyroxine on the synthesis and secretion of
apoB by normal rat hepatocytes: The results are given in table (30) and 't' values are given in table (30a).

The addition of hormone \((10^{-6} \text{M})\) in vitro caused significant increase in the secretion of \((^{3}\text{H})\)-apoB into the medium as well in the cell associated apoB by hepatocytes isolated from normal rat liver.

2. Pulse chase experiment: Cells were pulsed with 100\text{µCi} of \((^{3}\text{H})\)-leucine for 3 hours. The medium was removed, the cells were reincubated with the medium containing nonradioactive leucine and incubated for 4 hours in presence of thyroxine \((10^{-6}\text{M})\).

Results are given in table (31).

There was significant increase in the release of apoB into the medium in the presence of thyroxine.

3. The effect of hypothyroidism on the synthesis and secretion of apoB:

(a) Secretion of apoB into the medium:- Results are given in table (32) and 't' values are given in table (32a).

Hepatocytes isolated from livers of rats administered thiouracil showed significantly more apoB in the medium when
Table. 30

Incorporation of $({^3}H)$-leucine into apoB - Effect of thyroxine

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\ldots\ldots[CPM/mg\ cell\ protein\times 10^{-3}]\ldots\ldots$</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>22.82±1.75</td>
<td>22.93±1.68</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>41.75±2.21$^a$</td>
<td>26.34±1.36$^b$</td>
</tr>
</tbody>
</table>

Hepatocytes were incubated with $({^3}H)$-leucine (50µCi) in presence of thyroxine ($10^{-6}$ M) for 12 hours. The radioactivity associated with secreted and cell associated apoB was measured. The results given are the mean of four different experiments ± SD. On comparison with control,

a = $p < 0.01$

b = $p$ between 0.01 and 0.5

No symbol - not significant.

Table. 30a

$'t'$ values

<table>
<thead>
<tr>
<th>$'t'$ between control &amp; hormone</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine</td>
<td>14.14</td>
<td>3.16</td>
</tr>
</tbody>
</table>
Table. 31

Incorporation of (\(^{3}\)H)-leucine into apoB – Pulse chase analysis in presence of thyroxine

<table>
<thead>
<tr>
<th>Media</th>
<th>[CPM/mg cell protein]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>650±48</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>1166±110</td>
</tr>
</tbody>
</table>

The cells were pulsed with 100\(\mu\)Ci of (\(^{3}\)H)-leucine for 3 hours and then chased in presence of thyroxine for 4 hours. The radioactivity associated with secreted apoB was measured. The values given are the average of triplicate analysis ± SD.
Table. 32
Synthesis and secretion of (3^H)-apoB by hepatocytes isolated from experimental animals of different thyroid status

<table>
<thead>
<tr>
<th>Group</th>
<th>Media [CPM/mg cell protein]</th>
<th>Cell layer [mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4611±630</td>
<td>392±84</td>
</tr>
<tr>
<td>II</td>
<td>7554±670</td>
<td>1143±180</td>
</tr>
<tr>
<td>III</td>
<td>5992±710^b</td>
<td>660±120^a</td>
</tr>
</tbody>
</table>

The cells isolated from livers of the experimental animals were incubated with 20μCi of (3^H)-leucine for 12 hours. The radioactivity associated with secreted and cell associated apoB was measured. The results given are the mean of four different experiments ± SD.

When values of group II were compared with that of group I p<0.01 in both cases. When values of group III were compared with group II

a = p<0.01
b = p between 0.01 and 0.05

Table. 32a
't' values

<table>
<thead>
<tr>
<th>'t' between groups</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I &amp; II</td>
<td>6.4</td>
<td>7.56</td>
</tr>
<tr>
<td>II &amp; III</td>
<td>3.3</td>
<td>6.59</td>
</tr>
</tbody>
</table>
compared to controls. The animals administered thiouracil +
thyroxine showed decreased secretion of apoB into the medium when
compared with that given thiouracil alone.

(b) Cell associated apoB:- Results are given in table (32)
and 't' values are given in table (32a).

Hepatocytes isolated from thiouracil administered rats showed
increase in cell associated (3H)-apoB when compared to control rats.
Rats administered thyroxine along with thiouracil showed decrease in
cell associated (3H)-apoB when compared to those administered
thiouracil alone.

Section IV

Effect of catecholamines and cyclic AMP on the synthesis and
secretion of apoB by hepatocytes

Very little information is available on the direct effect of
catecholamines on the synthesis and secretion of apoB although the
importance of this hormone in regulating lipid metabolism under
conditions of acute stress is well recognized288. Short term
treatment of isolated liver preparations with epinephrine and
norepinephrine has been reported to rapidly suppress the secretion of
VLDL associated TG and cholesterol respectively289,290. Suppression
of VLDL-TG secretion by epinephrine in primary cultures of rat hepatocytes has been reported by Muramo et al. Kempen et al. also observed inhibition of the secretion of VLDL-TG by addition of dibutyryl cyclic AMP. But no report seems to be available on the in vitro addition of epinephrine or cyclic AMP on the synthesis and secretion of apoB by hepatocytes. In view of these, the effect of addition of epinephrine and cyclic AMP on the synthesis and secretion of apoB by rat hepatocytes in culture was studied.

Results

1. Effect of addition of epinephrine on the synthesis and secretion of apoB by rat hepatocytes: Results are given in table (33) and 't' values are given in table (33a).

Epinephrine (10^{-6} M) caused significant reduction in the amount of apoB in the medium when compared to control cells. There was also significant inhibition in the cell associated apoB in the presence of epinephrine.

2. Effect of in vitro addition of cyclic AMP: Results are given in table (33) and 't' values are given in table (33a).
Incorporation of $^{3}\text{H}$-leucine into apoB - Effect of epinephrine and $\text{Bt}_2\text{cAMP}$

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\text{[CPM/mg cell protein x 10}^{-3}\text{]}$</td>
</tr>
<tr>
<td>Control</td>
<td>22.82±1.75</td>
<td>22.93±1.68</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>18.42±1.34$^a$</td>
<td>14.00±0.98$^a$</td>
</tr>
<tr>
<td>$\text{Bt}_2\text{cAMP}$</td>
<td>19.23±1.02$^b$</td>
<td>15.71±1.60$^a$</td>
</tr>
</tbody>
</table>

The cells were incubated with epinephrine ($10^{-6}$M) and $\text{Bt}_2\text{cAMP}$ ($10^{-4}$M) for 12 hours in medium containing 50μCi of $^{3}\text{H}$-leucine. The radioactivity with secreted and cell associated apoB was measured. The results given are the mean of four different experiments ± SD. On comparison with control,

\[a = p < 0.01\]
\[b = p \text{ between 0.01 and 0.05}\]

<table>
<thead>
<tr>
<th>'t' between control &amp; hormones</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>3.99</td>
<td>9.19</td>
</tr>
<tr>
<td>$\text{Bt}_2\text{cAMP}$</td>
<td>3.55</td>
<td>6.63</td>
</tr>
</tbody>
</table>
As in the case of epinephrine, there was significant reduction in the amount apoB in the medium and cell associated apoB in the presence of dibutyryl cyclic AMP ($10^{-4}$M) when compared to control cells.

Section V

Effect of in vitro addition of insulin and glucagon on the synthesis and secretion of apoB by rat hepatocytes

Glucagon has been reported to inhibit the secretion of VLDL-TG in isolated preparations of rat liver. Currently there is no information on the effect of glucagon on apoB secretion from rat hepatocytes. In HepG2 cells, it has been reported that glucagon has no effect in VLDL secretion. Thus, whether glucagon plays a physiological role in regulating hepatic VLDL output is uncertain at present. Available reports on the effect of insulin on the synthesis and secretion of VLDL have been described briefly in the introduction. In view of the long-established lipogenic role of insulin, until recently it was assumed that the hormone acted directly on the liver to promote the secretion of VLDL. But insulin administration to human subjects has been reported to decrease hepatic VLDL output. In rats hepatic VLDL secretion rates in vivo were reported to be higher when plasma insulin levels
Studies with rat hepatocytes and human hepatoma (HepG2) cells have also consistently shown a direct inhibitory effect of insulin during a period of exposure shorter than 24 hours. Insulin is believed to promote the synthesis of TG but in short term exposure it is reported to prevent the secretion of VLDL from the hepatocytes. Insulin therefore appears to uncouple the synthesis of TG from its secretion. There is also some evidence that there is an impairment of apoB association with TG for secretion, although it has been suggested that insulin does not affect the expression of apoB gene in HepG2 cells. Pulse chase experiments have shown that in presence of insulin, a large proportion of newly synthesised apoB is degraded intracellularly rather than secreted.

In contrast to the inhibitory effect of short term insulin exposure, when the hormone is present for periods longer than 24-48 hours, the secretion of VLDL is enhanced.

In view of the contradictory reports on the effect of insulin on the synthesis and secretion of apoB, it was found desirable to confirm the effect of in vitro addition of this hormone on the synthesis and secretion of apoB by hepatocytes in culture. The effect of in vitro addition of glucagon was also studied and the results are discussed in this section.
Results

1. **Effect of addition of glucagon on the synthesis and secretion of apoB by rat hepatocytes:** The results are given in table (34) and 't' values are given in table (34a).

   Significant inhibition of the secretion of apoB into the medium was observed with the presence of glucagon ($10^{-6}$ M). Cell associated apoB also showed significant decrease in the presence of the hormone.

2. **Effect of in vitro addition of insulin on the synthesis and secretion of apoB by rat hepatocytes:** Results are given in table (34) and 't' values are given in table (34a).

   In vitro addition of insulin into the medium caused a significant decrease in the apoB secreted into the medium when compared to control cells. On the other hand, cell associated apoB showed a significant increase.

Discussion

The results now obtained indicate that hormones play a significant role in the synthesis and secretion of apoB by rat hepatocytes. The effect on the synthesis and secretion of apoB is
Table. 34
Incorporation of \( \textsuperscript{3}H \)-leucine into apoB - Effect of glucagon and insulin

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Media [CPM/mg cell protein]</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2457±168</td>
<td>744±38</td>
</tr>
<tr>
<td>Glucagon</td>
<td>1606±150</td>
<td>588±40</td>
</tr>
<tr>
<td>Insulin</td>
<td>1680±144</td>
<td>992±61</td>
</tr>
</tbody>
</table>

The cells were incubated with glucagon \( (10^{-6}\text{M}) \) and insulin \( (10^{-6}\text{M}) \) for 12 hours in medium containing \( 20\mu\text{Ci} \) of \( \textsuperscript{3} \text{H} \)-leucine. The radioactivity associated with secreted and cell associated apoB was measured. The results given are the mean of four different experiments ± SD. On comparison with control \( p<0.01 \) in all cases.

Table. 34a
't' values

<table>
<thead>
<tr>
<th>'t' between control &amp; hormones</th>
<th>Media</th>
<th>Cell layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon</td>
<td>7.56</td>
<td>5.65</td>
</tr>
<tr>
<td>Insulin</td>
<td>7.02</td>
<td>6.91</td>
</tr>
</tbody>
</table>
found to vary with different hormones, some exert a stimulatory effect while others exert an inhibitory effect.

Dexamethasone and corticosterone cause stimulation of the secretion of apoB into the medium by the hepatocytes. There is increased synthesis of apoB also by hormones as is evident from the increase in the total apoB. Many previous reports in this respect relate to secretion of VLDL-TG, and cholesterol. A recent report of Martin-Sanz indicated increased secretion of apoB in the presence of dexamethasone. The increased secretion of apoB may be associated with the increased secretion of VLDL-TG and this stimulatory effect on VLDL secretion may contribute to the well established hypertriglyceridemia associated with high circulating levels of adrenal glucocorticoids.

The results obtained with p-estradiol also indicate a stimulatory effect for this hormone on the synthesis and secretion of apoB. The results now obtained agree with the previous reports which relate to increased VLDL-TG secretion by hepatocytes isolated from estrogen treated female rats. Few reports seem to be available on the in vitro effect of this hormone on apoB synthesis and secretion by hepatocytes, although it has been reported that the apo VLDL-II mRNA in liver showed significant increase on estrogen administration to rooster. It has also been reported that estrogen induces apoB
in premenopausal women. In human hepatoma cells (HepG2 cells) estrogen at a concentration of 20nM caused no significant increase in apoB. Subsequent studies indicate that the concentration of hormone from 15-250nM has no additional effect on the rate of accumulation of apoB. But when the initial concentration of estrogen is increased to 500nM, the rate of accumulation of secreted apoB and apoE approximately doubled.

Thyroxine in vitro in the present study stimulated both the synthesis and secretion of apoB. The literature on the effect of thyroid hormone is extensive and confusing and in many cases contradictory. Few previous reports seem to be available on the in vitro effect of this hormone on the synthesis and secretion of apoB. Most of the studies in this respect refer to the synthesis and secretion of VLDL by hepatocytes isolated from hypothyroid or hyperthyroid animals. The results obtained on the effect of thyroid hormone under in vitro conditions appear to differ from in vivo conditions. Suppression of thyroid hormone by thiouracil administration significantly increased the synthesis and secretion of apoB and administration of thyroxine counteracted this stimulatory effect. The results now obtained are in agreement with the report of liver perfusion studies that there was decreased secretion of VLDL by hyperthyroid rats. On the other hand they differ from those reported by Dolphin & Forsyth that hypothyroid rats secreted less VLDL. The present results on the effect of the hormone in vivo are
also in agreement with those obtained by Keyes et al.\textsuperscript{172} that livers from hyperthyroid rats secreted decreased amounts of VLDL and incorporated less \textsuperscript{14}C-\textsuperscript{oleate} into VLDL-TG.

Epinephrine and cyclic AMP inhibited the synthesis and secretion of apoB. On the other hand, \textsuperscript{3}estradiol and thyroxine stimulated the synthesis and secretion of apoB. Very little information is available on the direct effect of catecholamines on the synthesis and secretion of apoB. But the available report that short term treatment of isolated liver preparations with adrenaline or noradrenaline rapidly suppressed secretion of VLDL-TG is in agreement with our results.\textsuperscript{289} The other two hormones which show inhibitory effects on the synthesis and secretion of apoB, are insulin and glucagon. While glucagon showed decrease in the cell associated apoB also, insulin in vitro caused accumulation of apoB in the cell.

Long term effects of insulin were not investigated in the present study. The suppression of glucagon on VLDL synthesis and secretion agree with the inhibitory effect of cyclic AMP, since this hormone acts via cyclic AMP. The results now obtained with glucagon are also in agreement with the previous reports that glucagon inhibits VLDL-TG secretion in hepatocytes. No previous information is available on the effect of this hormone on apoB synthesis and secretion by normal hepatocytes, but in HepG2 cells, it has been reported that this cell line is insensitive to this hormone.\textsuperscript{292}
The results discussed above demonstrate that hormones have a significant role in the synthesis and secretion of apoB containing lipoproteins by primary cultures of rat hepatocytes. The effect of hormones on apoB synthesis and secretion as lipoproteins can be a direct one through regulatory effects at the translational or transcriptional level of synthesis of apoB. There are a number of reports in support of this possibility.

It has been reported that thyroid hormone directly modulates growth hormone gene expression at the level of transcription and stabilises mRNA of several hepatic genes concerned with lipogenesis\textsuperscript{297,298}. Davidson et al\textsuperscript{299} recently reported that thyroxine administration to hypothyroid rats induces a shift in the production of apoB, from B\textsubscript{100} to B\textsubscript{48}. This is due to the modulation of thyroid hormone in introducing a stop codon in the rat liver B\textsubscript{100} mRNA. It is not known whether thyroxine is involved in this post-transcriptional process also.

A number of hormones have been reported to enhance general protein synthesis. There are also a number of cases where specific protein synthesis is also influenced by hormones, examples include casein gene expression by lactogenic hormones, ovalbumin induction by estrogen etc\textsuperscript{300,301}. It is possible that the effects of hormones on apoB production observed here may be a direct effect. In the case
of hormones where the general protein synthesis was enhanced, it was found that the increase obtained in response to the respective hormones in the case of apoB was more than the increase in the rate of incorporation of the precursor amino acids in the total TCA precipitable protein, indicating that the effects of hormones on apoB may be specific. Similarly, in the case of hormones where apoB synthesis was reduced, it was not on account of any effect on the general protein synthesis.

Another possibility is that the hormones through second messengers such as cyclic AMP may modulate apoB production. Evidence in support of this comes from the observation that hormones and drugs which increase intracellular level of cAMP caused a decrease in incorporation of radioactive precursors into apoB. Cyclic AMP and hormones such as glucagon which increase intracellular level of cAMP have been shown to inhibit cholesterogenesis and output of VLDL in a number of cases. Decreased availability of cholesterol can lead to decreased synthesis of apoB. But in the case of insulin which causes a decrease in the cAMP levels, a decrease in apoB is also observed in short term experiments. But in long term experiments there was a significant increase in the production of VLDL, possibly indicating that other factors may also contribute to insulin mediated effect on apoB production.
It is also possible that the hormones may affect the intracellular Ca\(^{2+}\) mediated events. Disruption of cellular calcium homeostasis with calcium ionophores has been shown to result in increased apoB secretion,\(^{195}\) whereas calcium antagonists have been reported to inhibit apoB production\(^{194}\). This observation is particularly relevant as Ca\(^{2+}\) is involved in almost all the secretory processes\(^{303}\). It has also been shown that in CaCo2 cells, calcium ionophores caused a dose dependent increase in apoB secretion while apoA-I was not affected indicating specific effect of calcium ions on apoB secretion\(^{195}\). Moreover, these effects could be mediated by calmodulin antagonists\(^{195}\). There are a number of reports showing the effect of hormones on intracellular Ca\(^{2+}\) levels\(^{304,305}\). It is therefore possible that the effects of some of the hormones observed here on apoB synthesis and secretion may be mediated through intracellular calcium ions. Epinephrine has been shown to increase intracellular cAMP levels as well as to affect intracellular Ca\(^{2+}\) mobilization\(^{304}\).

It is also possible that effects of hormones on apoB production is secondary to their effect on other metabolic pathways particularly that of lipids. Results discussed in the previous chapters have shown the possible role of lipids particularly cholesterol in the synthesis and secretion of apoB by hepatocytes. Therefore, any effect of hormones on lipid metabolism may have an
indirect effect on apoB production. There are reports showing that different hormones affect the metabolism of lipids. Triiodothyronine has been shown to stimulate fatty acid and cholesterol synthesis in cultured rat hepatocytes\textsuperscript{306}. Simonet \\& Ness\textsuperscript{307} reported that triiodothyronine increases HMG-CoA mRNA half life by stabilizing it.

Glucocorticoids have been shown to increase VLDL-TG output from liver and decreased oxidation of free fatty acids to ketone bodies. It has been reported that there exists a reciprocal relationship between hepatic triglyceride synthesis and ketogenesis\textsuperscript{308}. Phosphatidate phosphorylase, an important regulatory enzyme of triglyceride synthesis has been shown to be activated by glucocorticoids in vitro and in vivo\textsuperscript{309-311} which can result in increased availability of triglycerides.

Thus it appears that hormones which increase production of apoB also have been reported to increase cholesterogenesis and/or lipogenesis, while hormones and drugs which reduce apoB production have been shown to decrease the synthesis of lipids. These observations suggest the possibility that hormones may also be exerting an indirect effect on apoB production through their effect on the metabolism of lipids and cholesterol.
It is pertinent to note that the effect of hormones discussed above refers to in vitro studies. The in vivo effects of these hormones in certain cases have been shown to be different \(^{170,171,173,287}\). Hormones in general appears to have diverse functions in different tissues and their effects in certain studies are synergistic while in certain other cases are antagonistic \(^{156,164}\).

Thus the results discussed above indicate that hormones play an important role on the metabolism of apolipoprotein B in hepatocytes. But the precise molecular mechanism through which the hormones influence the metabolism of apoB is not clear.