

## Basic Sciences in Medicine

# HEPATOMA McARDLE-RH7777 CELLS HAVE THE SAME RESPONSE AS NORMAL RAT HEPATOCYTES TO BOTH DIBUTYRYL-cAMP AND ANTICALMODULIN W-7

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### ABSTRACT

The effects of cAMP-analogue dibutyryl-cAMP and anticalmodulin W-7 were studied on *de novo* synthesis and secretion of lipids in cultures of hepatoma McArdle RH7777 cells and normal rat hepatocytes. Dibutyryl-cAMP and W-7 separately caused a significant decrease in the secretion of *de novo* synthesized triacyl [<sup>3</sup>H]glycerol in both cultures of McArdle cells and rat hepatocytes. The inhibitory effects of dibutyryl-cAMP and W-7 were concentration-dependent and appeared at the lowest concentration examined, 5 μM and 20 μM respectively. Dibutyryl-cAMP at a concentration of 50 μM and W-7 (20 μM) suppressed the secretion of triacylglycerol by approx. 38% ( $p < 0.05$ ) and 37% ( $p < 0.05$ ) respectively. Dibutyryl-cAMP but not W-7 also suppressed the secretion of phosphatidylcholine significantly. Dibutyryl-cAMP and W-7 had no significant effect upon [<sup>3</sup>H]glycerol-labeled *de novo* formed triacylglycerol and phosphatidylcholine, except at the highest concentration tested, 500 μM and 50 μM respectively, where both triacylglycerol and phosphatidylcholine synthesis were suppressed significantly. Similar findings were obtained on cultured hepatocytes.

The molar ratios of newly made triacylglycerol/phosphatidyl choline in the media and cells of hepatocytes were about four times compared to that of McArdle cells, indicating more lipidation and core expansion of nascent lipoprotein particles in hepatocytes. The molar ratio of triacylglycerol/phosphatidylcholine in the medium and cells of cultured McArdle cells was unchanged significantly in the presence of either dibutyryl-cAMP or W-7. However, the molar ratio was decreased 25% ( $p < 0.01$ ) in the presence of dibutyryl-cAMP in the media but not in the cells of cultured hepatocytes. These results suggest that hepatoma McArdle

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ported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.

**Abbreviations used:** Apo apolipoprotein, BSA: bovine serum albumin. Bt<sub>2</sub>-cAMP: dibutyryl cyclic adenosyl monophosphate, Ch: cholesterol, CE: cholesteryl ester, DMEM: Dulbecco's modified Eagles medium, ER: endoplasmic reticulum, PC: phosphatidylcholine, TG: triacylglycerol, VLDL: very low density lipoprotein.

cell's response to both cAMP-analogue and calmodulin antagonist are comparable to that of normal rat hepatocytes. In addition, the inhibitory effects of dibutyryl-cAMP and W-7 at low concentration are unlikely to be due to the suppression of triacylglycerol and phosphatidylcholine synthesis.

*MJIRI, Vol. 15, No. 4, 209-217, 2002.*

**Keywords:** Calmodulin, Cyclic AMP, Hepatocyte, McArdle cells and VLDL

## INTRODUCTION

The atherogenic nature of apoB containing lipoproteins has already been demonstrated.<sup>1,2</sup> Therefore, understanding the factors that govern the regulation of assembly, synthesis and secretion of very low density lipoprotein (VLDL) associated components are important. The metabolism of VLDL in the liver is controlled by hormonal and metabolic regulations and recently has been reviewed.<sup>1-6</sup> Secretion of VLDL is not only suppressed by calcium-linked agents such as catecholamines,<sup>7-10</sup> prostaglandins,<sup>11</sup> and calcium antagonists,<sup>12-13</sup> but also by agents which act via the cyclic AMP (cAMP) pathway including glucagon,<sup>14-16</sup> cAMP derivatives<sup>16-18</sup> and cAMP dependent protein kinase.<sup>16</sup>

By now, most data on hepatic metabolism of lipoproteins are obtained on cultured rat hepatocytes<sup>6-16</sup> and perfused rat liver.<sup>17</sup> However, recently, several immortalized cell lines derived from normal rat and human hepatocytes are used extensively to study the assembly and secretion of apoB containing lipoproteins.<sup>3,6</sup> The signal transduction pathways are modified in cancer and malignant cells and extensive attempts have been made to correlate the malignancy nature of hepatomas to this modification.<sup>19,20</sup> The metabolism of cAMP is altered and regulated differently in many cancers and malignant cell lines relative to normal liver tissues.<sup>20</sup> The responsiveness of different signal transduction to different stimuli are changed and varied in different hepatoma cell lines.<sup>19</sup> The low level of cAMP in rat hepatoma cell lines H35 and MH1C1 appears to result from a combination of unstimulated adenylate cyclase and apparently elevated phosphodiesterase activities.<sup>20</sup> However opposite results are also presented.<sup>19</sup> Human hepatoma cell line HepG2 cells lack the smooth endoplasmic reticulum (ER) and also the cAMP-linked signaling pathway. Ginsberg et al. used a cAMP-analogue to stimulate triacylglycerol hydrolysis in HepG2 cells irrespective of its other effects.<sup>21</sup> HepG2 and rat hepatoma McArdle RH7777 cells secrete apoB containing lipoproteins at a less rate and higher density.<sup>3,4,20,22</sup>

It is reported that in hepatoma cells compared to normal rat hepatocytes the cAMP-pathway is attenuated while the pathway of calcium/calmodulin is more sensi-

tive to stimuli.<sup>19-21</sup> The results collected on hepatoma cell lines may be due to the modified nature of hepatomas and hence is not capable of extending to normal hepatocytes. However, long term viability of hepatoma cells and reproducibility of the results collected on different cell preparations make hepatoma cell lines a convenient tool for studying apoB containing lipoprotein metabolism.<sup>6</sup> The long-term objective of the series of works is to study the effects and interaction of two signal transduction pathways, cAMP and calcium/calmodulin, on VLDL assembly and secretion. In the present investigation, the effects of cAMP-analogue and anticalmodulin W-7 were examined on *de novo* synthesis and secretion of lipids in cultured hepatoma McArdle cells and normal rat hepatocytes. The results showed that McArdle cells responded to both signal transduction pathways and the results are comparable to that of normal rat hepatocytes.

## MATERIAL AND METHODS

### Materials

Dibutyryl-cAMP (D-0260), bovine serum albumin, (BSA, essential fatty acid-free), collagenase 330U/mg (C-5138) and W-7 (N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide) (A-3261) were purchased from Sigma Chemical Company. [1(3)-<sup>3</sup>H]glycerol (2.6Ci/mmole) was obtained from Amersham, Canada (Oakville, Ontario, Canada). DMEM, sodium pyruvate, penicillin/streptomycin, fetal bovine and horse sera were from Gibco BRL (Life Technologies Inc., Grand Island, NY). TLC plates were prepared from Whatman Ltd. (NJ). All other chemicals and solvents were of reagent or better quality and were obtained from local suppliers.

### Growth of McArdle cells

Wild typed McArdle RH7777 cells obtained from ATCC were cultured in 60 mm-diam. petri dishes with DMEM containing pyruvate, antibiotics, 10% (v/v) fetal bovine serum and 10% (v/v) horse serum. When the cells were about 70% confluent, the medium was changed to serum free DMEM experimental medium as described in the legend of figures. All cultures were maintained in 100mm-diam. dishes (Corning) at 37°C in humidified air

(90% saturation) containing 5% CO<sub>2</sub>.<sup>23</sup>

### Hepatocyte preparation, culture and incubation

L<sub>6</sub>-hepatocytes were isolated in sterile condition from male Spargue-Dawley rats (150-200g) by two steps collagenase method. In brief, rat liver was perfused in situ by Ca<sup>2+</sup> free Hank's saline buffer containing EGTA (0.5 mM) for 1 min, then with Hank's solution containing Ca<sup>2+</sup> (2 mM) and collagenase (200 IU/mL) for 9 min.<sup>23</sup> Isolated hepatocytes were suspended in Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal calf serum and centrifuged two times at 50 g for 2min. The final cell suspension was counted in the presence of 0.04% trypan blue. Exclusion of the dye was >90% in all preparations. Cells were plated in DMEM containing 20% calf serum in collagen-coated (87µg/mL) 60mm-diam. sterile petri dishes for 6h in an atmosphere of air/CO<sub>2</sub> (19:1) Afterwards, medium and non-adherent cells were discarded and adherent cells washed with 3×2mL of fresh DMEM. Cells were incubated in 2mL of DMEM containing oleate/BSA (0.3mM/0.5%, v=5) and other drugs as described in the legends of figures. At the end of the incubation period, the cells were cooled to 4°C on ice and the medium was collected. The cells were washed with ice cooled phosphate-buffered saline (PBS) and removed from the dish as described previously.<sup>23</sup> Cells and media were analyzed for assays of lipids and protein.<sup>24,25</sup>

### Lipid analysis

At the end of incubations, media were separated from the cells and cells were washed with ice-cold phosphate-buffered saline, harvested in the same buffer and dispersed by brief sonication. Cellular and media lipids were extracted according to Folch et al. in the presence of non-labeled lipids carrier.<sup>23</sup> The lipids were applied to thin-layer chromatography plates and developed to 1/3 the height with chloroform/methanol/acetic acid/water (25: 15: 4: 2 by v/v) to separate glycerophospholipids, followed by development in heptane/isopropyl ether/acetic acid (60: 40: 4 by v/v) to separate neutral lipids.<sup>23</sup> The lipid spots were visualized by exposure to iodine, bands corresponding to various lipid classes were scraped and the associated radioactivity was determined by scintillation counting.

### Other methods

Protein concentration was determined by the method of Bradford by the Bio-Rad protein assay kit using BSA as a protein standard. Oleate/BSA complex was prepared by the following method of Heimberg et al.<sup>17,28,31</sup> Oleic acid was neutralized by equimolar and 5% excess KOH (0.1N) and then added to a solution of BSA in DMEM at room temperature and then sterilized by filtration. The final concentration of oleate/BSA was 0.3mM/0.5% (mo-

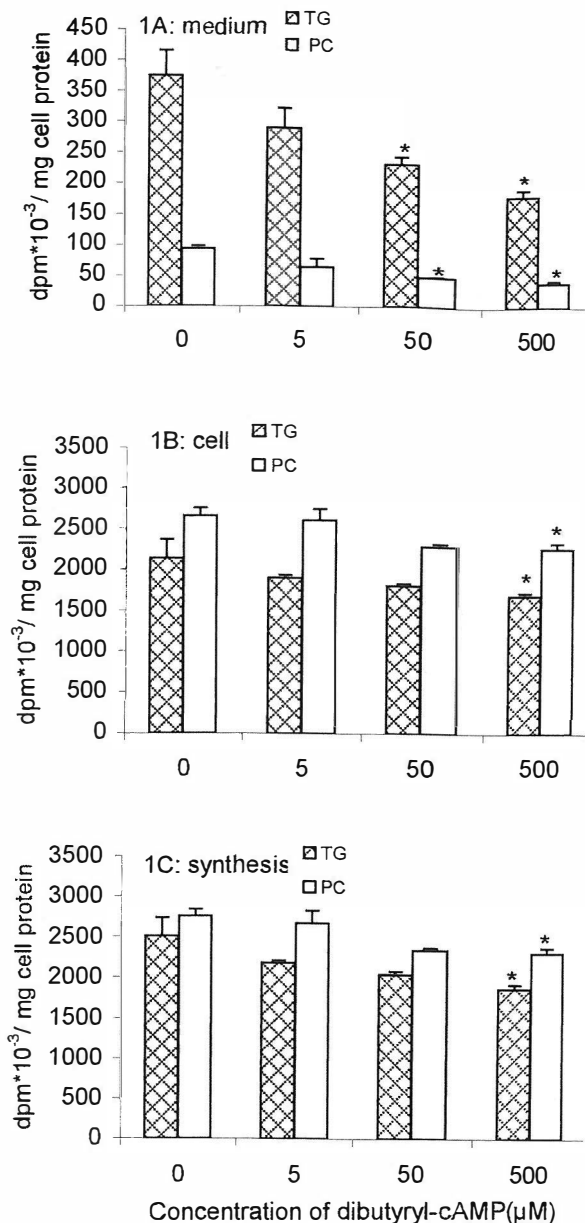


Fig. 1. Concentration-response curves for the effects of Bt<sub>2</sub>cAMP on secretion, cellular level and *de novo* synthesis of cultured McArdle cells. Wild type McArdle- RH7777 cells at 70% confluency were incubated 2h in DMEM containing oleate/BSA (0.3mM/0.5%) and [<sup>3</sup>H]glycerol (5µCi/mL) in the presence of different concentrations of Bt<sub>2</sub>cAMP (0,5,50,500µM) in the *Material and Methods* section. The data represent A) secretion of *de novo* made TG (hatched bars) and PC (plain bars), B) cellular levels of *de novo* made TG and PC and C) *de novo* synthesis of TG and PC. The results are presented as the means±SEM of two interassays performed at least in three different cell preparations. All samples are compared to the control.\* Indicates that the corresponding value is significantly different from its respective control at the *p*≤0.05 confidence level. Error bars for several determinations are too small to be seen.

lar ratio of oleate to BSA is:  $v=5$ ).

#### Statistical analysis

The significant differences between samples and corresponding control were accessed by t-student test. The results are presented as the means  $\pm$  SEM of two interassays performed at least in three different cell preparations.

### RESULTS

#### Concentration-response curves for the effects of $Bt_2$ -cAMP on *de novo* synthesis and secretion of lipids in cultures of McArdle cells.

Since it has been reported that the cAMP transduction pathway is attenuated in hepatoma cells relative to normal hepatocytes, we obtained concentration-response curves for the effects of  $Bt_2$ -cAMP on *de novo* synthesis and secretion of lipids in cultured wild type McArdle cells. McArdle cells at 70% confluency were incubated 2h in DMEM containing oleate/BSA (0.3mM/0.5%) and [ $^3H$ ]glycerol (5 $\mu$ Ci/mL) in the presence of varying concentrations of  $Bt_2$ -cAMP (0.5, 50, 500 $\mu$ M).  $Bt_2$ -cAMP suppressed secretion of *de novo* made TG in a concentration-dependent manner (Fig. 1A). The  $Bt_2$ -cAMP concentration needed to reveal a biological response was 50 $\mu$ M. At this concentration, the suppression of TG secretion was approx. 38% ( $p \leq 0.05$ ). The secretion of PC was also affected 30% ( $p \leq 0.05$ ).  $Bt_2$ -cAMP had no significant effect on cellular content of [ $^3H$ ]glycerol-labeled TG and PC, except at the highest concentration examined, 500 $\mu$ M (Fig. 1B). The overall rate of *de novo* TG and PC synthesis was calculated from the sum of the labeled medium and cellular TG of PC when cells are cultured in the presence of exogenous [ $^3H$ ]glycerol (Fig. 1C).  $Bt_2$ -cAMP had no significant effect on *de novo* synthesis of TG and PC, except at the highest concentration used (500 $\mu$ M), where TG and PC synthesis was suppressed by 26% ( $p \leq 0.05$ ) and 16% ( $p \leq 0.004$ ) respectively. The decrement in TG secretion observed at low concentrations of  $Bt_2$ -cAMP was unlikely to be due to change in *de novo* synthesis of TG or PC.

#### Concentration-response curves for the effects of anticalmodulin W-7 on *de novo* synthesis and secretion of lipids in cultures of McArdle cells (Fig. 2A, B, C)

It is reported that the calcium/calmodulin transduction pathway is more sensitive to stimulation in hepatoma cells compared to normal hepatocytes. We obtained concentration-response curves for the effects of W-7 on *de novo* synthesis and secretion of lipids in cultured wild typed McArdle cells. McArdle cells at 70% confluency were incubated 2h in DMEM containing oleate/BSA

(0.3 $\mu$ M/0.5%) and [ $^3H$ ]glycerol (5 $\mu$ Ci/mL) in the presence of different concentrations of W-7 (0.5, 10, 20, 50, 100  $\mu$ M). W-7 suppressed secretion of *de novo* made TG in a concentration-dependent manner. W-7 at 20 $\mu$ M inhibited secretion of TG by about 37% ( $p \leq 0.05$ ). W-7 had no significant effect on PC secretion except at more than 20mM concentration, when PC synthesis is inhibited significantly. W-7 had no significant effect on cellular content of [ $^3H$ ]glycerol-labeled TG and PC (Fig. 2B) and on *de novo* synthesis of TG and PC (Fig. 1C), except at more than 20 $\mu$ M concentration. At 50 $\mu$ M concentration of W-7, TG and PC synthesis were suppressed by 20% ( $p < 0.05$ ) and 30% ( $p < 0.01$ ) respectively. The reduction of TG secretion observed at a low concentration of W-7, where TG and PC synthesis were unaffected, was unlikely to be due to change in *de novo* synthesis of TG or PC.

#### Effect of $Bt_2$ -cAMP and W-7 on *de novo* synthesis and secretion of lipids in cultured rat hepatocytes (Fig. 3A, B)

Hepatocytes were isolated from rats and incubated 6h in DMEM containing 20% fetal bovine serum and then media and detached cells were removed. Hepatocytes were washed twice and incubated for 2h in 2mL serum-free DMEM containing oleate/BSA (0.3 $\mu$ M/0.5%) and [ $^3H$ ]glycerol (5 $\mu$ Ci/mL) in the absence (control) or presence of  $Bt_2$ -cAMP (100 $\mu$ M) or W-7 (20mM). At the end of the incubation period, media were collected and cells were washed with PBS and cells and media were extracted and analyzed as described in the experimental section. Dibutyl-cAMP at a concentration of 100mM inhibited secretion of TG by 38% ( $p < 0.05$ ) and also PC by 17%, but not significantly ( $p < 0.16$ ). Dibutyl-cAMP had no significant effect on *de novo* synthesis of TG and PC. W-7 at 20 $\mu$ M suppressed TG secretion by approx. 30% ( $p < 0.05$ ) but had no significant effect on secretion of PC and synthesis of TG and PC (results not shown). Neither dibutyl-cAMP nor W-7 had significant effect on the cellular content of *de novo* made TG and PC.

#### The molar ratio of newly synthesized TG/PC in the medium and cells of cultured McArdle cells and rat hepatocytes

The predicted ratio of TG/PC is that anticipated for incorporation of radioactive glycerol into TG and PC if only one pool of glycerol exists for their synthesis. The molar ratio of newly synthesized TG/PC in medium and cells of McArdle was  $4.04 \pm 0.61$  and  $0.81 \pm 0.06$ , and the ratio in medium and cells of the hepatocytes was  $18.7 \pm 0.43$  and  $3.80 \pm 0.26$  respectively. Therefore, the molar ratio of TG/PC has increased about 4-times in media and cells of normal hepatocytes relative to

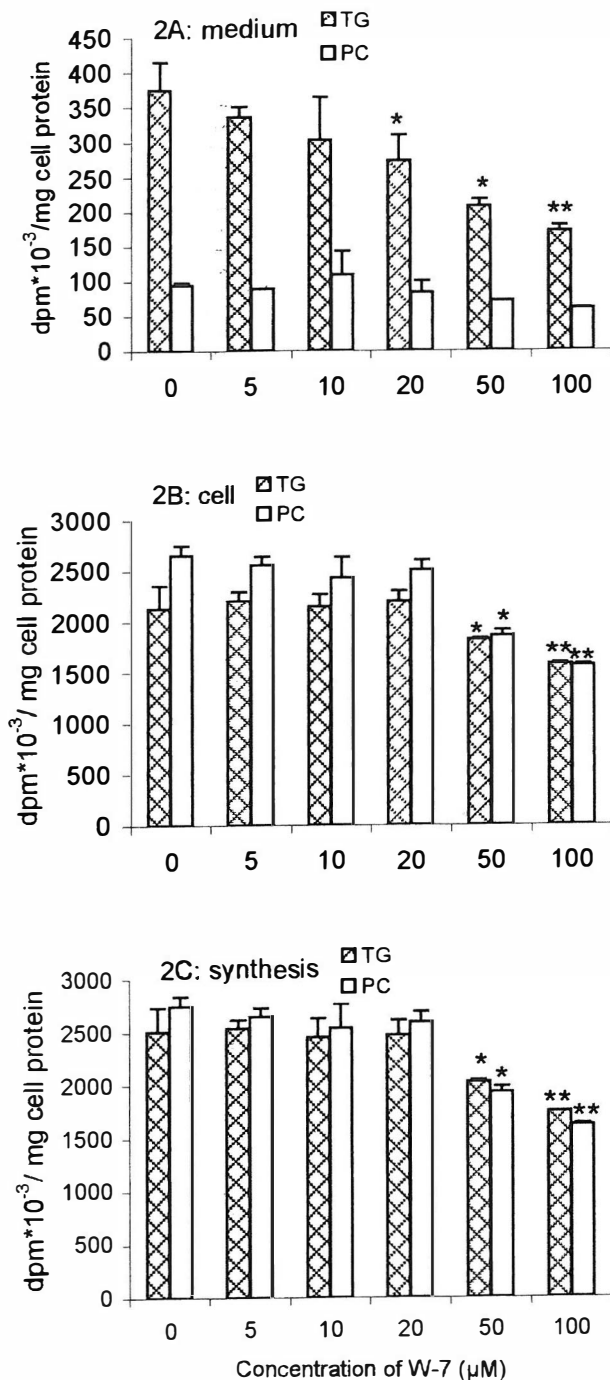


Fig. 2. Concentration-response curves of the effects of anticalmodulin W-7 on secretion, cellular level and *de novo* synthesis of lipids in cultured McArdle cells. Wild type McArdle- cells at 70% confluence were incubated 2h in DMEM containing oleate/BSA (0.3mM/0.5%) and [<sup>3</sup>H]glycerol (5μCi/mL) in the presence of different concentrations of W-7 (0, 5, 10, 20, 50, 100μM) as described in the *Material and Methods* section. The data represent: A) secretion of *de novo* made TG and PC, B) cellular levels of *de novo* made TG and PC and C) *de novo* synthesis of TG and PC. The results are presented as the means±SEM of two interassays performed at least in three different cell preparations. All samples are compared to the control. \* and \*\* indicate  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

McArdle cells. The molar ratio of TG/PC in the medium and cells of cultured Mc-Ardle cells was unchanged significantly in the presence of either Bt<sub>2</sub>-cAMP or W-7. However, the molar ratio was decreased by 25% ( $p < 0.01$ ) in the presence of dibutyryl-cAMP in the media but not in the cells of cultured hepatocytes.

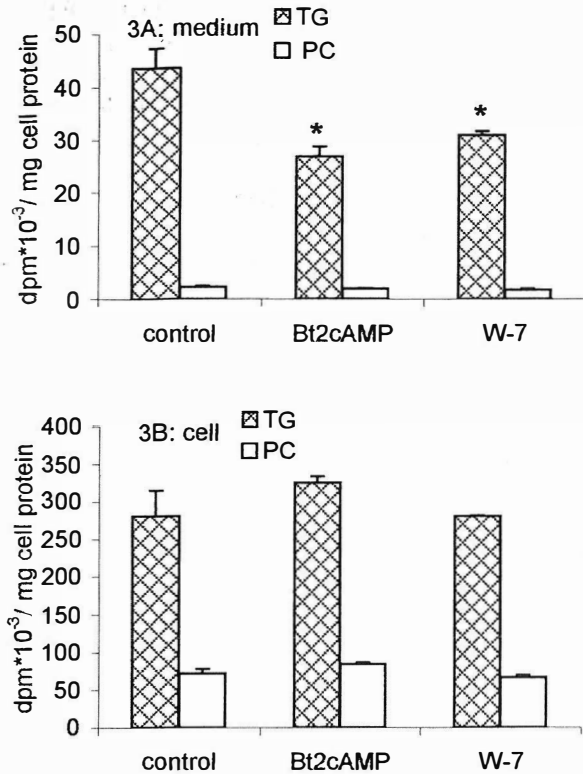
## DISCUSSION

The metabolism of apoB containing lipoproteins in hepatic cells is under control of both cAMP and calcium/calmodulin pathways. In spite of the reports implying that the cAMP pathway is modified in hepatoma cells, the data presented here revealed that the cAMP analogue dibutyryl-cAMP inhibits TG secretion at the same concentration needed in normal rat hepatocytes. The low level of cAMP in hepatoma cell lines is related to less adenylate cyclase and more phosphodiesterase activity.<sup>20</sup> However none is eligible to dibutyryl-cAMP, since it is not produced by adenylate cyclase and is not susceptible to hydrolysis by phosphodiesterase.<sup>17</sup> Our findings show that the significant effect of dibutyryl-cAMP on secretion of *de novo* made TG in cultured McArdle cells appeared at a concentration that is seen in cultured rat hepatocytes<sup>14-16</sup> and perfused rat liver.<sup>17</sup> Gibbons et al. have shown that activation of the cAMP pathway via protein kinase-A inhibits directly or indirectly the secretion of apoB containing lipoproteins in cultures of hepatocytes.<sup>16</sup> Inhibition of TG secretion occurred in a situation in which TG and PC synthesis were unaffected. It has also been reported that dibutyryl-cAMP at a low concentration unchanged cholesterologenesis.<sup>16,18</sup> Dibutyryl-cAMP could not influence the overall rate of TG synthesis, except at the highest concentration tested. Hence the inhibitory effect of cAMP at low concentration on TG secretion is not attributed to inhibition of TG, PC and cholesterol synthesis. There is a dual reciprocal control over the synthesis of TG and oxidation of fatty acids.<sup>16</sup> Dibutyryl-cAMP inhibits *de novo* synthesis of fatty acids and channeling it to oxidation versus the esterification pathway. Inhibition of *de novo* synthesis of fatty acids and shifting exogenous fatty acids to the oxidative pathway are probable mechanisms to diminish esterification of [<sup>3</sup>H]glycerol to TG and PC and subsequently their secretion.

Anticalmodulin W-7 unexpectedly inhibited secretion of *de novo* made TG in both cultured hepatoma McArdle cells and normal rat hepatocytes. This finding about the net effect of calmodulin antagonist is in agreement to some reports<sup>27,34</sup> and is opposite to others.<sup>8,10,30</sup> Many investigators reported that calmodulin antagonists used alone had no significant effect.<sup>9,10,30</sup> However, our results and the results presented by others indicate that



## Response of McArdle RH7777 Cells to Dibutyryl-cAMP



**Fig. 3. The effects of Bt<sub>2</sub>cAMP and W-7 on secretion, cellular level and *de novo* synthesis of lipids in cultured rat hepatocytes.** Hepatocytes were isolated from rats and incubated 6h in DMEM containing 20% FBS and then media and detached cells were removed. Cells were incubated for 2h in DMEM containing oleate/BSA (0.3mM/0.5%) and [<sup>3</sup>H]glycerol (5μCi/mL) in the absence (control) or presence of Bt<sub>2</sub>cAMP (100μM) or W-7 (20μM). At the end of the incubation period, cells were washed with PBS and cells and media were analyzed as described in the Experimental section. The data represent: **A)** secretion of *de novo* made TG and PC, **B)** cellular levels of *de novo* made TG and PC. Results are expressed as the means±SEM of three experiments. All samples are compared to the control. \*Indicates *p*<0.05.

calmodulin antagonists have a net effect. It is reported that the pathways of calcium/calmodulin and cAMP have been modified in hepatoma cells relative to normal hepatocytes.<sup>19,20</sup> In hepatoma cell lines, the calcium/calmodulin pathway is more sensitive, while the cAMP signaling pathway is less sensitive to stimulation.<sup>19-20</sup> Thus, we have done similar experiments on cultured rat hepatocytes to exclude the possibility that, existence of a response to W-7 may be due to the modified nature of hepatoma cells. However, similar results were obtained

on both hepatoma cells and normal hepatocytes. Therefore, the assumption that the response of McArdle cells to calmodulin antagonist is related to the modified nature of hepatoma cells may be ruled out.

W-7 at all concentrations examined here suppressed TG secretion; however, different mechanisms are probably involved at varying concentrations of W-7. At a W-7 concentration equal or less than 20μM, where TG and PC synthesis were unaffected, the effect is unlikely attributed to TG and PC synthesis. By now, there is no clear explanation for the mechanism of the effects of low concentrations of W-7 on TG secretion. The effect of W-7 may be related to inhibition of calmodulin. Available knowledge about the role of calcium/calmodulin in the regulation of hepatic VLDL metabolism is too limited. Intraluminal calcium of ER is required for proper folding and translocation of nascent apoB through the secretory pathway.<sup>9-13</sup> Cytosolic calcium is the trigger of microtubule-dependent exocytosis.<sup>7</sup> Increments of cytosolic calcium lead to activation of exocytosis in all cells except in hepatocytes that lead to inhibition of VLDL-associated triacylglycerol.<sup>17</sup> Therefore, it seems that these two requirements are opposite to each other. Calcium mobilizing agents release Ca<sup>2+</sup> from intracellular stores (predominantly ER) to the cytosol. Depletion of intraluminal Ca<sup>2+</sup> stores prevents secretion of apoB associated components, and at the same time the increment in cytosolic Ca<sup>2+</sup> leads to activation of the calcium/calmodulin pathway.<sup>10,25</sup> But there is less data about the role of the latter in the metabolism of apoB containing lipoproteins in the liver.

Other mechanisms also may be involved. Anticalmodulin drugs have side effects. The side effects of calmodulin antagonists are related to nonspecific binding to other calcium-binding proteins (enzymes, receptors) and different membranes (plasma and HR membranes).<sup>29</sup> Calmodulin antagonists bind to the hydrophobic domain of calmodulin, and the binding is calcium-dependent at low concentrations of antagonist.<sup>29</sup> The selectivity of binding of antagonists to calmodulin was abolished at 10μM or greater concentrations of antagonist, since antagonists bind to other calcium-binding proteins. Unfortunately, by now it is not possible to choose the appropriate concentration for only anticalmodulin activity. Indeed many actions of calmodulin antagonists on membrane structure and hormone receptors occur at much lower concentrations than needed to block calmodulin.<sup>29</sup> However, the role of calcium/calmodulin remains to be determined. It is reported that, W-7 in cultured fibroblasts and HepG2 cells enter the cells and via interaction with Δ<sup>24</sup>-reductase, an enzyme involved in cholesterol synthesis inhibits *de novo* synthesis of cholesterol.<sup>34</sup> By extending this mechanism to liver cells, it

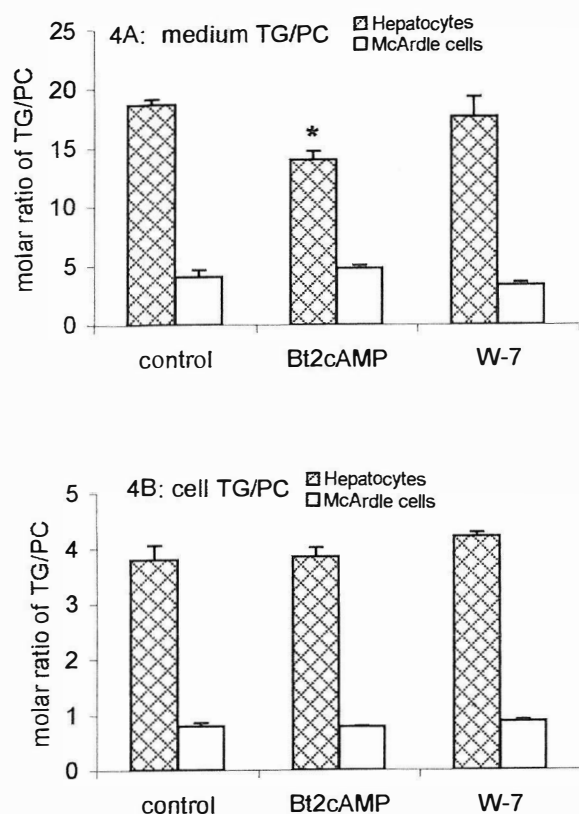
can be deduced that W-7 at low concentration inhibits secretion of newly made TG via inhibition of *de novo* synthesis of cholesterol. However, we will examine this mechanism on cultured hepatoma cells and normal rat hepatocytes in the future.

The results presented here show that W-7 inhibits secretion of newly synthesized TG at concentrations of W-7 at which TG and PC synthesis are unaffected. We also examined higher concentrations of W-7, and the calmodulin antagonist inhibited TG and PC synthesis at a concentration equal or greater than 50  $\mu$ M. Antagonists internalize into the cell and attach to the surface of the endoplasmic reticulum and as an amphiphilic cation change its surface charge to be more positive.<sup>31</sup> This can regulate the subcellular partition of membrane bonded enzymes. Such a regulation has been reported for phosphatidate phosphohydrolase (PAP)<sup>31,32</sup> and CTP phosphorylcholine cytidyltransferase.<sup>33</sup> Bridley et al. have shown that chlorpromazine, as an amphiphilic cation, at concentrations of 50  $\mu$ M or more dislocates PAP from ER to the cytosol and subsequently inhibits it.<sup>28,31-2</sup> Dislocation of PAP from the ER membrane to the cytosol leads to inhibition of TG and PC synthesis. Vance et al. also reported that chlorpromazine has a direct effect on cytidyltransferase and inhibits PC synthesis.<sup>33</sup> Calmodulin antagonists also interfere with many lipids as activators of many kinases such as protein kinase-A and -C.<sup>29</sup> Phenothiazines and W-7 as amphiphilic cations have a direct effect on cell membrane physical characteristics which are probably related to their lipophilic properties.<sup>29</sup> However, these mechanisms that correspond to the inhibition of TG and PC synthesis are not involved at concentrations of less than 20  $\mu$ M of antagonist.

Our data also show that when cells are labeled in the presence of exogenous [<sup>3</sup>H]glycerol, the molar ratio of newly made TG/PC secreted as the medium of cultures of hepatocytes was ~20 and of McArdle cells was ~4. These results agree with that reported on cultured hepatocytes<sup>14-6</sup> and hepatoma cells.<sup>21-3,34</sup> The increased molar ratio of *de novo* synthesized TG/PC in normal hepatocytes relative to hepatomas indicates more lipidation and core expansion of nascent lipoprotein. The availability of TG for lipoprotein assembly greatly influences the size of the apoB containing lipoproteins.<sup>21</sup> There are several models for lipoprotein assembly and secretion.<sup>1-6</sup> In sequential assembly, lipidation of nascent apoB takes place cotranslationally in rough ER and leads to lipid-poor particles. Additional core TG is added through the secretory pathway, particularly at the smooth ER, the major site of TG synthesis. The molar ratio of TG/PC increases during transport through the secretory pathway. Newly made TG, however, can enter either a pool near its site of synthesis on the surface of the ER or enrich the cyto-

plasmic TG pool. The distribution of newly made TG between these two pools determines the amount of TG available for lipoprotein assembly and secretion.<sup>3,14,21</sup> The microsomal TG pool which resides mainly in the smooth ER is small but very active and has a more regulatory role on assembly of lipoproteins. It has been shown that about 2% and 30% of newly made TG is directed to the microsomal pool in hepatoma cells and hepatocytes respectively.<sup>14,21,23</sup> The stored TG does not seem to be transferred directly to the developing lipoprotein particle; instead, they undergo lipolysis followed by re-esterification.<sup>23</sup> Now, there are two possibilities to explain for the difference between assembly and secretion of lipoproteins in hepatoma relative to normal hepatocytes. First, it is reported that smooth ER is essentially absent in hepatoma cells, thus the transfer of new TG into developing lipoproteins is limited by physical limitation of the site of lipidation, i.e., smooth ER.<sup>21</sup> Therefore, more newly formed TG is directed to the cytosolic pool in hepatomas (~98%) compared to hepatocytes (~70%).<sup>21</sup> Second, lipolysis of cytosolic TG is done by a newly discovered triacylglycerol hydrolase (TGH) characterized recently by this laboratory.<sup>23,35</sup> We also recently showed that microsomal TGH is absent in hepatoma HepG2 and McArdle cells.<sup>35</sup> The lack of microsomal TGH limits TG availability for assembly of lipoproteins. Thus, the limited physical site for addition of newly formed TG and absent microsomal TGH activity in hepatoma cells limits lipidation and core expansion of nascent particles. The lipid-poor particles secreted in hepatoma cells are thought to be product of the early addition of TG in the rough ER with little or no further significant addition of core lipids prior to secretion. Hence, hepatomas secrete LDL-size and not VLDL-size particles. The results presented here also show that W-7 has no influence upon the molar ratio of TG/PC in the media or on cells of both cultured McArdle cells and rat hepatocytes. However dibutyryl-cAMP caused a considerable decrease in the molar ratio of TG/PC in the media of cultured hepatocytes. The reason for this may be the enhanced fatty acid oxidation and ketogenesis in the presence of dibutyryl-cAMP, but compounds which affect calcium homeostasis, i.e. W-7, do not stimulate ketogenesis.<sup>13</sup>

The results presented here reveal that rat hepatoma McArdle cells respond to both cAMP-analogue and anticalmodulin W-7 and the response was comparable to that of normal rat hepatocytes. Most information regarding hepatic synthesis and secretion of the apoB containing lipoproteins has been collected from studies performed using cultured hepatocytes or hepatoma cell lines. Primary hepatocytes are often the preferred model system not only because they synthesize and secrete the whole spectrum of lipoproteins but also because these



**Fig. 4.** The molar ratio of newly synthesized triacylglycerol/phosphatidylcholine in the medium and cells of cultured McArdle cells and rat hepatocytes. The situation of incubations of McArdle cells and hepatocytes are as described in the legend of Figures 1 and 3. The molar ratio of newly made TG/PC is calculated as the ratio of incorporation of [ $^3\text{H}$ ] glycerol (dpm) into fractions TG and PC. The molar ratio of newly synthesized TG/PC in the medium (**A**) and cells (**B**) of McArdle cells (plain bars) and of hepatocytes (hatched bars) are shown. Results are expressed as the mean $\pm$ SEM of three experiments. All samples are compared to the control. \*Indicate  $p < 0.01$ .

cells have retained responsiveness to most physiological stimuli. However, using hepatocytes are limited because of the lack of reproducibility between various hepatocyte preparations and short term viability of the cells. Thus, immortalized hepatoma McArdle cells have been a valuable alternative to primary hepatocytes.

## ACKNOWLEDGEMENT

This work was supported by the grants from the Canadian Institute of Health Research and the Alberta Heritage Foundation for Medical Research. Dr. Lehner is an Alberta Heritage Foundation for Medical Research

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