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SUPPRESSION OF VLDL-TRIACYLGLYCEROL SECRETION BY BOTH α- AND β-ADRENOCEPTOR AGONISTS IN ISOLATED RAT HEPATOCYTES

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ABSTRACT

The effects of alpha and beta-adrenergic stimulation on triacylglycerol secretion were investigated in isolated rat hepatocytes. Epinephrine within 3h of incubation suppressed triacylglycerol secretion by 35% and increased its cellular content by 18%. The inhibitory effect of epinephrine was abolished by inclusion of phentolamine and also prazosin but not with propranolol. Trifluoperazine concealed the inhibitory effect of epinephrine in a dose-dependent manner, whereas theobromine did not have any significant effect. The secretion of triacylglycerol was suppressed not only by the α-agonist phenylephrine but also by the β-agonist isoproterenol. Dibutyryl-cyclic AMP also inhibited secretion of triacylglycerol by approximately 30%. The results indicate that epinephrine suppressed triacylglycerol secretion via the α_1-adrenoceptor whereas stimulation of beta–as well as alpha–adrenoceptors can exert a similar effect. Calcium-calmodulin dependent protein kinase may be involved in the down-regulation of VLDL secretion. The unexpected effect of isoproterenol has been discussed in relation to "dual signaling" and also the "store-dependent calcium entry" hypotheses.

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INTRODUCTION

Synthesis, assembly and secretion of VLDL associated components are subject to hormonal and metabolic regulations and recently have been reviewed.[1-6] Secretion of VLDL is not only suppressed by calcium-linked agents such as catecholamines,[7-9] prostaglandins,[10] and calcium antagonists,[11,12] but also by agents that act via the cAMP pathway including glucagon,[13-15] cAMP deriviatives [15-17] and cAMP-dependent protein kinase.[15] Although secretion of VLDL is regulated by both signal transduction systems (i.e., calcium and cAMP pathways), epinephrine acts mainly through the α_1-adrenoceptor in the liver.[18]

Calcium may be involved in the secretion of VLDL through one or more of at least four processes: I) Synthesis of secretory products. Calcium-mobilizing agents regulate the activities of the enzymes involved in glycerolipid biosynthesis.[19-21] Alpha-adrenergic stimulation inhibits lipogenesis and leads to channeling of fatty acid substrate from esterification to oxidation pathway.[22] II) Post-transcriptional modifications and subsequent translocation through the secretory pathway. Intraluminal calcium is required for the actions of signal peptidase, chaperone and apoprotein-B (apo-B) itself.[4] The optimum concentration of Ca^{2+} within the endoplasmic reticulum (ER) may be essential for proper folding, assembly and secretion of nascent apo-B.[4,23] This is supported by the actions of prostaglandins and also verapamil that selectively inhibit VLDL secretion but have no influ-

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ence on any parameters of hepatic lipid metabolism.[10] III) Regulation of microtubules and microfilaments, and IV) Fusion of secretory vesicles to the plasma membrane. Secretion of VLDL is achieved via a general mechanism for protein secretion and is microtubule dependent, and is inhibited by colchicin.[24] The increment in cytosolic calcium in the liver cells in contrast to some other secretory cell types, has been accompanied with an inhibition of the secretory process.

In the present study, the effects of various agents effective to adrenoceptors have been investigated on triacylglycerol secretion in rat hepatocytes. The data indicates that epinephrine suppresses triacylglycerol secretion via the α_1-adrenoceptor, whereas both α- and β-agonists can lead to a similar effect.

MATERIALS AND METHODS

Chemicals

Dibutyryl adenosine 3′,5′-cyclic monophosphate (Bt_2-cAMP), (-)epinephrine, oleic acid, bovine serum albumin (essentially fatty acid free) and trifluoperazine were obtained from Sigma (USA). Collagenase (5000 U/mg protein) was purchased from Merck (Germany), prazosin from Pfizer (USA) and theobromine from BDH (England). The sources of all other chemicals were as previously described.[25,26,34]

Hepatocyte isolation

Hepatocytes were isolated from Spargue-Dawley rats weighing 250-300 g that had free access to laboratory chow and water. L_4-hepatocytes were isolated by two-step collagenase perfusion technique as described previously.[26] Trypan blue exclusion was greater than 90% and less than 10% of total LDH was released.

Hepatocyte incubation

Hepatocytes (8±0.5mg protein/mL) were incubated for 3h at 37°C in a total volume of 4 mL of Krebs-Ringer bicarbonate (KRB) containing 0.5% (w/v) bovine serum albumin, 0.25 mM oleate, 20 mM glucose and 2.5 mM $CaCl_2$ in siliconized flasks shaking at 90 cycles/min under an atmosphere of O_2: CO_2(19:1).

Lipid analyses

Incubation was stopped on ice and the medium removed after centrifugation at 1500 g for 3 min. The medium was centrifuged at 12000 g for 10 min at 4°C to remove broken cells and cell debris and the total volume of the supernatant was extracted for lipid analysis according to Folch et al.[27] Manipulative losses of triacylglycerol during extraction were accounted for by addition of glycerol [^{14}C]-trioleate as internal standard. The recovery of glycerol [^{14}C]-trioleate was determined by scintillation counting.[14] The cell pellet was washed twice by ice cold hemogenization solution[28] and sonicated by an ultrasonic processor and extracted for lipid analysis as described previously.[26] The mass of triacylglycerol was measured by using a kit purchased from Zist-Shimi, Iran (Triglycerides GPO-PAP). VLDL secretion was estimated by measuring the appearance of triacylglycerol in the incubation medium as it is well established that more than 95% of medium triacylglycerol is in the form of VLDL.[28] The dependence of secreted triacylglycerol to VLDL fraction was assessed by precipitation and ultracentrifugation methods as described by Mangiapane & Brindley.[28]

Other analytical procedures

Protein concentration was determined by the method of Lowry et al.[29] LDH was assayed by a colorimetric method, using a diagnostic kit (Sigma).

Statistical analysis

The results were expressed as mean±S.E.M. The statistical significance of any observed differences was tested by Student's t-test.

RESULTS

The effect of epinephrine on triacylglycerol secretion in the absence and the presence of α-adrenoceptor antagonists are shown in Fig. 1. The mass of triacylglycerol was measured in the lipid extract of the incubation medium. The rate of triacylglycerol secretion was 1.58±0.09 (μmole/3h/g wet liver) in the absence of epinephrine. Epinephrine at 10μM concentration suppressed triacylglycerol secretion by about 35% (p<0.005). The inhibitory effect of epinephrine was abolished in the presence of 10μM phentolamine, a general α- antagonist. Trifluoperazine, an anticalmodulin drug, abolished the effect of epinephrine in a dose dependent manner by about 50% at 10μM and completely at 20μM.

The effect of epinephrine on triacylglycerol secretion in the absence and the presence of cAMP-linked agents is shown in Fig. 2. The values for triacylglycerol secretion for control and epinephrine-treated samples are as in Fig. 1. The inhibitory effect of epinephrine has remained unchanged in the presence of propranolol (PRO, 10μM). In addition, theobromine (TB, 1μM), a cAMP-phosphodiesterase inhibitor, did not have any significant effect on epinephrine action. Dibutyryl-cAMP (Bt_2-cAMP, 0.1 mM) also inhibited triacylglycerol secretion alone by approx. 30% (p<0.01).

The effect of epinephrine, phenylephrine and isoproterenol on triacylglycerol secretion are depicted in Fig. 3. The rate of triacylglycerol secretion in the presence of phenylephrine (PE, 10μM) and isoproterenol (ISO, 10μM) was 1.17±0.07 and 1.23±0.09 (μ mole/3h/g wet liver), that implies 25% and 22% inhibition, respectively. None of the antagonists examined alone have any significant effect on triacylglycerol secretion (data not shown).

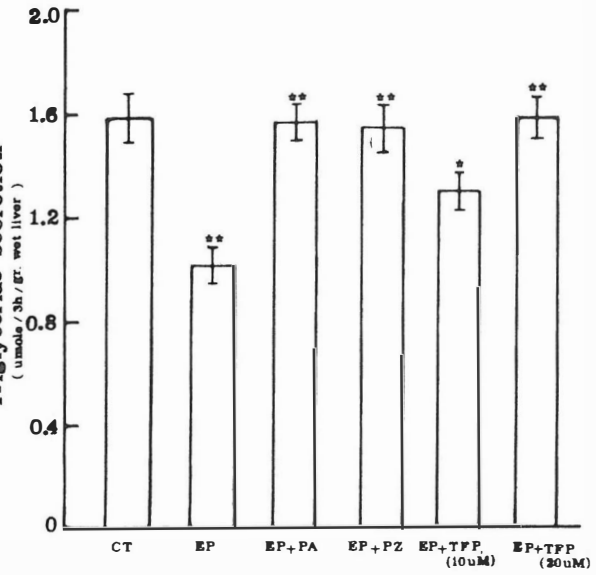

Fig. 1. Effects of epinephrine on triacylglycerol secretion in the absence and the presence of α-antagonists. Hepatocytes at a concentration of 8±0.5 mg protein/mL were incubated 3h in KRB containing 20μM-glucose and 0.25 μM-oleate in a total volume of 4mL at 37°C. Hepatocytes were preincubated for 5 min with 10 mM-phentolamine (PA), 1μM-prazosin (PZ) and 10, 20 μM-trifluoperazine (TFP). The rate of triacylglycerol secretion was expressed as μ mole TG/3h/g wet liver. It is assumed that 1μmole of triacylglycerol is 885 mg and one gram wet weight of liver is equal to 158 mg total cell protein. Results are expressed as the mean±S.E.M. of four interassays performed at least in three different cell preparations. The epinephrine treated sample was compared to the control and the others to the epinephrine treated one. *,** indicate $p<0.025$ and $p<0.005$, respectively.

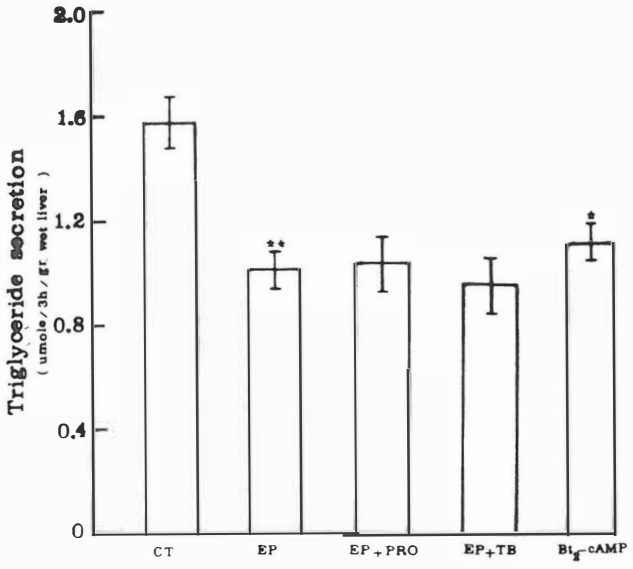

Fig. 2. Effects of epinephrine on triacylglycerol secretion in the absence and the presence of cAMP-linked agents. The values of triacylglycerol secretion of control and epinephrine treated samples are as in Fig. 1. Hepatocytes were preincubated for 5 min with 10 μM-propranolol (PRO) and 1 mM-theobromine (TB). All other experimental conditions are as described in the legend of Fig. 1. The epinephrine and dibutyryl-cAMP (Bt_2-cAMP, 0.1mM) treated samples are compared to control and the others to the epinephrine treated one. *,** indicate $p<0.01$ and $p<0.005$, respectively.

The effect of epinephrine on cellular triacylglycerol content is shown in Fig. 4. The mass of triacylglycerol was measured in the lipid extract of the cells. Hepatocyte triacylglycerol levels were 9.64±1.0 and 11.31±0.86 (μmole/g wet weight of liver) in the absence and presence of epinephrine respectively, which implies about 18% increment ($p<0.05$). The stimulatory effect of epinephrine has been antagonized by prazosin but retained in the presence of propranolol. In every case, the total triacylglycerol contents of the system (the sum of the cell and incubation medium) was almost constant.

DISCUSSION

The present results indicate that epinephrine decreases the secretion of triacylglycerol and increases its content in isolated rat hepatocytes. The observation that the inhibitory effect of epinephrine on triacylglycerol secretion was antagonized by a general α-adrenoceptor antagonist (phentolamine) and also by a specific α_1-antagonist (prazosin), but not with the β-antagonist propranolol suggests α_1-receptor involvement. This idea was also supported by the influence of trifluoperazine (TFP) –but not theobromine– on the action of epinephrine. α_1-Suppression of triacylglycerol secretion is in accordance with previous reports.[7-9] TFP is a competitive inhibitor of calmodulin and can attenuate signaling mediated by it.[30,31] Results presented here demonstrated that TFP concealed the inhibitory effect of epinephrine in a dose-dependent manner. Therefore, calcium-calmodulin multiprotein kinase (CaM-MPK) may be involved in the regulation of VLDL secretion. This is the first demonstration that activation of the calcium-calmodulin pathway is required for down-regulation of VLDL secretion. Microtubule associated protein-2 (MAP-2), Tau factor and probably tubuline itself have been shown to be phosphorylated by CaM-MPK.[31] Phosphorylation by CaM-MPK promotes the disassembly and inhibits the rate of ressembly of microtubules.[31] CaM-MPK also participates in α-adrenergic stimulation of ketogenesis.[22] Alpha-1 stimulation of hepatocytes is associated with a release of calcium from ER to the cytosol.[32] Release of intracellular calcium was found to be accompanied by calcium influx across the plasma membrane into the cytosol by a mechanism known as "store-dependent calcium entry".[33] Therefore, α_1-stimulation leads to the simultaneous depletion of calcium stores of ER and an increment in the cytosolic calcium concentration, both of which may cause suppression of VLDL secretion. Thus far, more attention has been focused on the former and it has been deduced that an optimum concentration of Ca^{2+} within

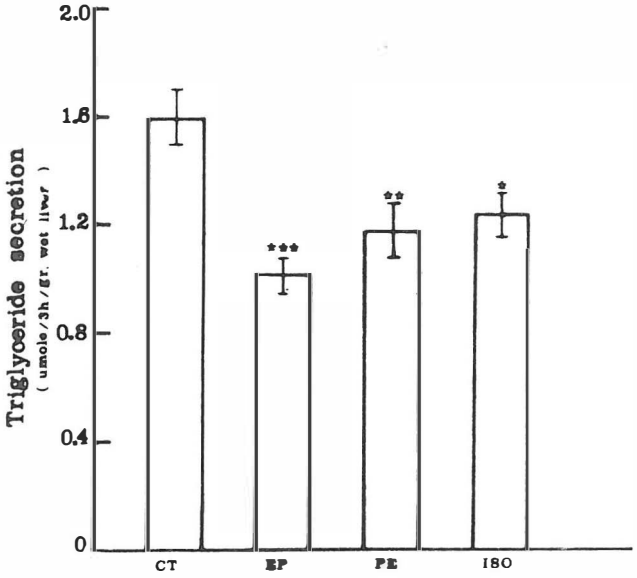

Fig. 3. Effects of epinephrine, α- and β-adrenoceptor agonists on triacylglycerol secretion. The values of control and epinephrine treated samples are as in Fig. 1. Hepatocytes were incubated 3h with epinephrine (EP, 10μM), phenylephrine (PE, 10μM), and isoproterenol (ISO, 10μM). Results are shown as mean±S.E.M. for at least three different cell preparations. All samples are compared to control. *p<0.025, **p<0.01, ***p<0.005.

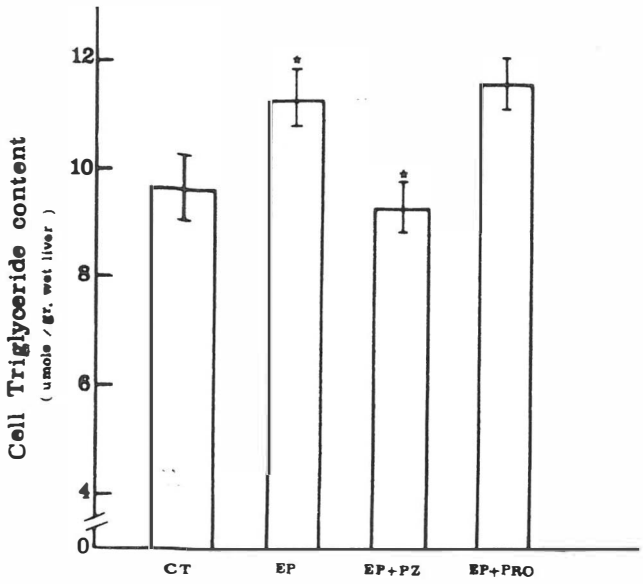

Fig. 4. Effects of epinephrine on cellular triacylglycerol content in the absence and presence of α- and β-antagonists. Hepatocytes were incubated for 3h in KRB as described in the legend of Fig. 1. Hepatocytes were preincubated for 5 min by 1μM- prazosin (PZ) and also 10μM-propranolol (PRO). Results are expressed as the mean±S.E.M. of four interassays performed at least in three different cell preparations. The epinephrine treated sample was compared to the control and the others to the epinephrine treated one. *p<0.05.

the ER may be essential for proper folding, translocation and secretion of apo-B.[4] But increasing cytosolic calcium via Ca^{2+}-calmodulin may also be involved in the inhibition of VLDL secretion. However, further investigation is required to elucidate the role of Ca^{2+}-calmodulin in the regulation of VLDL secretion.

The fact that the effect of epinephrine is mediated via the α_1-receptor does not necessarily exclude the involvement of cAMP in the regulation of VLDL secretion. In fact hepatic VLDL metabolism is regulated by both signal transduction systems.[7-17,34] The present data also shows that Bt_2-cAMP diminishes triacylglycerol secretion. This agrees with results obtained in perfused rat liver,[16] hepatocytes in incubation[17] and in culture.[15] By now, both calmodulin- and cAMP-dependent protein kinases may participate in suppression of VLDL secretion.

The most notable observation of the present investigation was the significant inhibition of triacylglycerol secretion, not only by the α-adrenoceptor agonist phenylephrine but also by the β- agonist isoproterenol. This is the first report for unexpected inhibitory effect of isoproterenol on VLDL secretion. A number of agents acting alone at a single receptor appear capable of generating more than one second messenger, i.e. dual signaling.[35] Glugacon[36-37] and isoproterenol[33,37] can raise both intracellular Ca^{2+} and cAMP in rat hepatocytes. Isoproterenol via activation of phospholipase-C (PLC) induces a rapid increase in inositol 1,4,5-triphosphate (IP_3) and consequently Ca^{2+}-mobilization.[37] It has been proposed that the effect of isoproterenol is achieved by activation of the β-adrenoceptor and mediated via different G-proteins, G_q and G_s, that lead to activation of PLC and adenylate cyclase, respectively.[35] Regulation of VLDL secretion by isoproterenol and glucagon may be instances in which both Ca^{2+} and cAMP act together. Cross-talking is present within and between the two signal transduction pathways, at receptors, G-proteins, effectors and second messengers.[35] The cAMP analogues and forskolin, an adenylate cyclase activator, also raised cytosolic calcium in rat hepatocytes.[37] Some evidence demonstrated that this was achieved by direct activation of PLC via G_q,[37] whereas interaction at further points is also probable.[35] Cyclic-AMP induces phosphorylation of the IP_3-receptor and modifies it more sensitive to IP_3.[33] At the present time, it seems that some of the effects of cAMP are exerted indirectly through affecting calcium homeostasis. However, whether the effects of activation of the cAMP pathway are exerted directly or at least partially indirectly via Ca^{2+} mobilization, and the points at which Ca^{2+} and cAMP interact to regulate VLDL secretion remains to be studied.

The results also indicate that epinephrine in the presence of propranolol increases the cellular triacylglycerol content significantly. This observation is in agreement with previous work[26] but nevertheless differs from the results reported by Brindle & Ontko.[7] The fact that, in the presence

of epinephrine, net accumulation of intracellular triacylglycerol was small, implies that it is exposed to intracellular degradation. In the previous work the time course of cellular lipids revealed that cellular triacylglycerol and phospholipid contents in the presence of epinephrine were higher than the control at all times, and the differences were constant along the time.[26] Since the differences were constant during the time course[26] and the total triacylglycerol content of the system (sum of the cell and incubation medium) were constant in the presence and absence of epinephrine, the accumulated lipids within the cells represent only those lipids that have not been secreted yet. Therefore, it seems that epinephrine inhibits triacylglycerol secretion via exertion of an inhibitory effect on the secretory pathway.

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