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α-Adrenergic suppression of very-low-density-lipoprotein triacylglycerol secretion by isolated rat hepatocytes

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The effect of adrenaline on triacylglycerol synthesis and secretion was examined in isolated rat hepatocytes. Cells were incubated with 0.5 mM-[1-^{14}C]oleate, and the accumulation of triacylglycerol and [^{14}C]triacylglycerol was measured in the incubation medium. Triacylglycerol appearing in the medium was present in a form with properties similar to very-low-density lipoproteins. Triacylglycerol, [^{14}C]triacylglycerol and [^{14}C]phospholipid contents of hepatocytes were also determined. Addition of 10 μM-(−)adrenaline decreased accumulation of glycerolipid in the incubation medium and also decreased cellular [^{14}C]phospholipid content. Prazosin abolished these effects, whereas propranolol did not. The hormone did not affect cellular triacylglycerol content or rates of incorporation of [1-^{14}C]oleate into cell triacylglycerol. The effect of adrenaline on the removal of newly secreted triacylglycerol and the secretion of synthesized glycerolipid was also examined. The catecholamine did not affect rates of removal of newly secreted triacylglycerol. Adrenaline did inhibit the secretion of pre-synthesized lipid by the cells, as assessed by the appearance of radiolabelled triacylglycerol from hepatocytes that had been preincubated with [1,2,3-^{3}H]-glycerol. Adrenaline did not affect rates of fatty acid uptake by hepatocytes, but did stimulate oxidation of [1-^{14}C]oleate, principally to $^{14}CO_2$.

INTRODUCTION

The liver plays a major role in the synthesis of triacylglycerol and its secretion into the blood in the form of very-low-density lipoproteins (VLDL). These synthetic and secretory processes have been shown to be responsive to several hormones, such as glucagon (Heimberg *et al.*, 1969; Soler-Argilaga *et al.*, 1978*b*; Kempen, 1980; Gibbons & Pullinger, 1987), insulin (Nikkila, 1974; Durrington *et al.*, 1982; Patsch *et al.*, 1983; Pullinger & Gibbons, 1985; Mangiapane & Brindley, 1986; Gibbons & Pullinger, 1987) and corticosteroids (Klausner & Heimberg, 1967; Cole *et al.*, 1982; Mangiapane & Brindley, 1986). In addition, data from some experiments *in vivo* suggest that catecholamines may modulate hepatic triacylglycerol and VLDL secretion (Nikkila, 1974; Chait *et al.*, 1979). In contrast with the hormones mentioned above, however, very little is known about the direct effects of catecholamines on hepatic triacylglycerol metabolism. Previous studies *in vitro* were conducted on the perfused rat liver (Heimberg & Fizette, 1963; Heimberg *et al.*, 1964). These experiments were complicated by the effects of the catecholamines on liver vasculature, which necessitated inclusion of an α-adrenergic antagonist in the perfusate. This antagonist would negate any effects on lipid metabolism that may be mediated via α-adrenoceptors.

Information on adrenergic modulation of lipoprotein secretion may be useful in understanding the humoral and neural regulation of this pathway, especially under conditions such as acute stress. It was the purpose of the present study, therefore, to investigate the direct effects of adrenaline, and particularly α_1-adrenoceptor stimulation, on hepatic triacylglycerol metabolism and secretion. By using the isolated hepatocyte system, vasoactive effects of the amines were avoided. In a preliminary study (Brindle & Ontko, 1986) we noted that adrenaline decreased accumulation of triacylglycerol in the incubation medium of hepatocytes incubated with 5 mM-glycerol.

MATERIALS AND METHODS

Chemicals

[1-^{14}C]Oleic acid and [1,2,3-^{3}H]glycerol were obtained from New England Nuclear Research Products (Boston, MA, U.S.A.). Collagenase was Worthington Biochemicals type CLS (from Cooper Biomedical, Malvern, PA, U.S.A.). Prazosin hydrochloride was a gift from Pfizer (Groton, CT, U.S.A.) and propranolol hydrochloride was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The sources of all other chemicals were as previously described (Zaleski & Ontko, 1985).

Hepatocyte preparation

Hepatocytes were isolated from male Holtzman rats (Sasco, Omaha, NE, U.S.A.) weighing 300–400 g. Rats had free access to Purina laboratory chow and water. Cells were isolated by perfusion of the liver *in situ* with medium (Krebs & Henseleit, 1932) containing 0.05% (w/v) collagenase, 5 mM-$CaCl_2$ and 20 mM-D-glucose. Metabolic integrity was assessed by measurement of ATP content (Dickson & Pogson, 1977).

Abbreviation used: VLDL, very-low-density lipoprotein.

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Hepatocyte incubations

Hepatocytes were incubated under an atmosphere of O_2/CO_2 (19:1) at 37 °C (5–7 mg dry wt./ml) in Krebs–Henseleit medium containing 2% (w/v) bovine serum albumin, 0.5 mM-oleate and 20 mM-D-glucose. Other substrates and hormones were included as indicated in the Results section. When present, the specific radioactivity of [1-^{14}C]oleate was 0.2 μCi/μmol and that of [1,2,3-^{3}H]glycerol was 1 μCi/μmol. Experiments on triacylglycerol synthesis and secretion were performed in silicone-treated 25 ml Erlenmeyer flasks with rubber stoppers, in a 4 ml volume. Incubations were shaken at 90 cycles/min. For lipid analyses, incubations were terminated by separating cells from medium by centrifugation (600 *g* for 10 min).

Oxidation experiments were performed in silicone-treated 20 ml scintillation vials in a volume of 2 ml. Incubations were terminated by addition of 0.2 ml of 3 M-$HClO_4$ through the rubber seal. CO_2 was collected in 0.25 ml of 10% (w/v) KOH in plastic centre wells, containing filter paper, suspended from vial stoppers. After CO_2 collection the acid-stopped incubations were centrifuged (2000 *g* for 10 min), and samples of the supernatant, containing water-soluble products, were neutralized with 0.5 M-triethanolamine/2 M-KOH. Radiolabelled water-soluble products were determined by liquid-scintillation counting of samples of supernatant after centrifugation (12000 *g* for 2 min) of neutralized extracts. ATP was determined by chemiluminescence (Stanley & Williams, 1969) in neutralized acid extracts of hepatocytes.

Secretion and re-uptake experiments

In these experiments, hepatocytes were incubated in a silicone-treated 250 ml conical flask under O_2/CO_2 (19:1) for 60 min (uptake experiments) or 45 min (secretion experiments). Each flask contained approx. 0.5 g dry wt. of hepatocytes in a volume of 80 ml of Krebs–Henseleit medium containing 2% (w/v) bovine serum albumin, 0.5 mM-oleate, 5 mM-[1,2,3-^{3}H]glycerol (1 μCi/μmol) and 20 mM-D-glucose. At the end of the respective incubation periods, hepatocytes were sedimented in ice-cold plastic tubes by centrifugation (50 *g* for 2 min). Cells were washed once, by resuspension in ice-cold medium containing 2% albumin and 20 mM-D-glucose, and re-centrifuged. In experiments in which secretion of prelabelled triacylglycerol was to be measured, cells were then incubated in 4 ml incubations containing 2% albumin, 20 mM-D-glucose and 5 mM-glycerol as previously described. Oleate was not included after pre-labelling, to minimize any new synthesis of triacylglycerol. In experiments in which triacylglycerol uptake was to be measured, cells were incubated for a further 45 min in 100 ml of incubation medium containing 2% albumin, 20 mM-D-glucose and 0.5 mM-oleate. After 45 min these cells were sedimented by centrifugation (1000 *g* for 2 min), and the incubation medium, now containing newly secreted [^{3}H]triacylglycerol, was used in incubations with hepatocytes isolated from a second rat. The [^{3}H]triacylglycerol content of this medium was determined at different times after the start of incubation in the presence and absence of adrenaline.

Lipid analyses

Lipids were extracted from samples of medium and cells as described by Folch *et al.* (1957). Water-soluble radioactivity was removed by washing once with water and either two (where [1-^{14}C]oleate was used) or six (where [^{3}H]glycerol was used) times with blank upper phase. Triacylglycerol from the medium was measured fluorimetrically (Chernick, 1969) and cellular triacylglycerol enzymically as previously described (Ide & Ontko, 1981). Samples of chloroform extracts were dried and lipid-associated radioactivity was quantified by liquid-scintillation counting. Lipid classes were separated by t.l.c. (Ide & Ontko, 1981).

Precipitation of VLDL

Apolipoprotein-B-containing lipoproteins were precipitated by the phosphotungstate method of Burstein *et al.* (1970), as described by Mangiapane & Brindley (1986).

Ultracentrifugation

VLDL were isolated from 15 ml of medium, collected after incubation, as previously described (Ide & Ontko, 1981). A 5 ml portion of NaCl ($d = 1.006$) containing 0.02% (w/v) EDTA and 0.01% thimerosal was layered over the incubation medium. This was subjected to ultracentrifugation at 105000 *g* for 22 h at 4 °C. The top 3.0 ml fraction was collected for lipid analysis.

RESULTS

Effects of adrenaline on accumulation of triacylglycerol in the incubation medium and cellular triacylglycerol synthesis

Addition of 10 μM-(−)adrenaline to hepatocytes incubated in the presence of [^{14}C]oleate markedly decreased rates of accumulation of triacylglycerol and [^{14}C]-triacylglycerol in the incubation medium (Table 1). This effect of the catecholamine was abolished by inclusion of the α_1-adrenoceptor antagonist prazosin, but not the β-antagonist propranolol. These data confirm our previous findings using hepatocytes incubated in the presence of [^{3}H]glycerol (Brindle & Ontko, 1986). Neither prazosin nor propranolol had any effects on amounts of lipid in the medium when added alone to the incubations (results not shown).

The effects of adrenaline on [^{14}C]oleate incorporation into cellular lipids and on hepatocyte triacylglycerol content are shown in Table 1. The hormone caused a slight decrease in incorporation of oleate into cellular lipid. This effect is a reflection of the marked inhibition of phospholipid synthesis observed on α_1-adrenergic stimulation of the hepatocytes (Table 1). Haagsman *et al.* (1984) have demonstrated α-adrenergic inhibition of phosphatidylcholine synthesis in isolated rat hepatocytes. As shown in Table 1, there was no significant effect of α_1-adrenergic stimulation on incorporation of radiolabelled oleate into cellular triacylglycerol, or in the triacylglycerol content of the hepatocytes.

Triacylglycerol secretion and removal

Accumulation of triacylglycerol in the incubation medium is the result of triacylglycerol secretion by hepatocytes and any degradation or re-uptake of the lipid that may occur under the conditions of these experiments. Adrenaline may therefore act to decrease triacylglycerol accumulation by suppressing output or stimulating degradation or uptake. The removal of newly secreted radiolabelled triacylglycerol from the

Table 1. Effect of (−)adrenaline in the absence and the presence of adrenergic antagonists on lipid accumulation in incubation medium and hepatocytes

Hepatocytes were incubated for 90 min in medium containing 0.5 mM-[1-^{14}C]oleate. When present, (−)adrenaline was added at 30 min to a concentration of 10 μM, and prazosin and propanolol were at 1 μM and 10 μM respectively. Rates of accumulation were measured between 30 and 90 min. Cellular triacylglycerol content was measured at 90 min. Results are expressed as a percentage of the control value, and are means ± S.E.M. for the numbers of preparations shown in parentheses. Absolute values are given in brackets for controls, as means ± S.E.M. Absolute values for triacylglycerol accumulation are expressed as nmol/h per mg dry wt. and for triacylglycerol content as nmol/mg dry wt. Radiolabelling data are given as d.p.m./h per mg dry wt. The significances of differences from control values were calculated by using a paired t test, and they are indicated by: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

	Value (% of control)				
	Medium		Cells		
Conditions	Triacylglycerol accumulation	[^{14}C]Triacylglycerol accumulation	Triacylglycerol content	[^{14}C]Triacylglycerol accumulation	[^{14}C]Phospholipid accumulation
Control	100 (7) [2.88 ± 0.32]	100 (7) [1429 ± 129]	100 (6) [38.1 ± 6.5]	100 (6) [2803 ± 427]	100 (6) [745 ± 64]
+ Adrenaline	56 ± 4*** (7)	56 ± 3*** (7)	103 ± 2 (6)	94 ± 5 (6)	47 ± 6** (6)
+ Adrenaline + prazosin	89 ± 7 (4)	94 ± 3 (4)	103 ± 2 (4)	91 ± 3 (4)	89 ± 5 (4)
+ Adrenaline + propranolol	47 ± 2** (4)	58 ± 5* (4)	102 ± 1 (4)	95 ± 8	47 ± 8* (4)

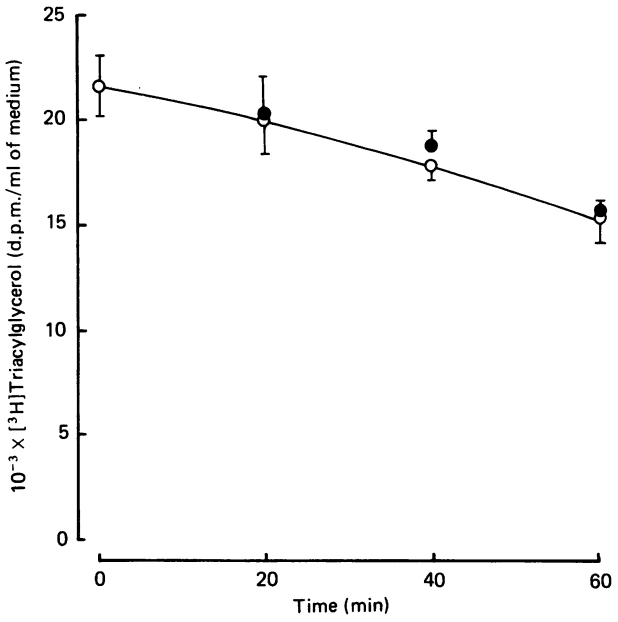

Fig. 1. Effect of (−)adrenaline on removal of newly secreted [^{3}H]triacylglycerol from the incubation medium

Hepatocytes were incubated in medium containing newly secreted [^{3}H]triacylglycerol (see the Materials and methods section) in the absence (○) or the presence (●) of 10 μM-(−)adrenaline. The hormone was added at 0 min. [^{3}H]-Triacyglycerol content of the medium was determined at the times indicated. Results are means ± S.D. for a single representative experiment.

incubation medium is depicted in Fig. 1; there was a time-dependent removal of newly secreted triacylglycerol in the presence of hepatocytes. Addition of adrenaline did not alter the rate of disappearance of lipid (Fig. 1). These data therefore imply that the catecholamine was acting by inhibiting triacylglycerol output from the hepatocytes

Triacylglycerol output may be modulated by affecting the incorporation of triacylglycerol into lipoproteins and/or the movement of these nascent lipoproteins to the cell surface and their release. In order to determine the influence of adrenaline on these processes, cellular triacylglycerol was pre-labelled by incubation of hepatocytes with [^{3}H]glycerol in the presence of oleate. The release of these pre-labelled (pre-synthesized) triacylglycerols was monitored during a subsequent incubation in the absence and presence of adrenaline (Fig. 2). Fig. 2 shows a time-dependent release of presynthesized triacylglycerol by the hepatocytes. Adrenaline inhibited secretion of pre-synthesized triacylglycerol by over 40%. This inhibition was observable even at the earliest, 20 min, time point.

Characteristics of secreted triacylglycerol

Treatment of samples of incubation medium with phosphotungstate caused precipitation of all the radioactively labelled triacylglycerol secreted. The phosphotungstate reagent precipitates apoprotein-B-containing lipoproteins (Burstein *et al.*, 1970). More than 70% of the secreted triacylglycerol was collected from the top fraction of $d = 1.006$ NaCl layer after ultracentrifugation. This is similar to the distribution of triacylglycerol found after ultracentrifugation of perfusate from perfused rat liver (Ide & Ontko, 1981). These results indicate that the secreted triacylglycerol was predominantly in the form of VLDL. This is in agreement with previous studies (Sundler *et al.*, 1973; Kempen, 1980; Haagsman & Van Golde, 1981), which demonstrate VLDL secretion by isolated rat hepatocytes in suspension.

Fatty acid uptake and oxidation

The [^{14}C]oleate content of the incubation medium measured at various times after start of incubation is depicted in Fig. 3. Adrenaline did not affect fatty acid

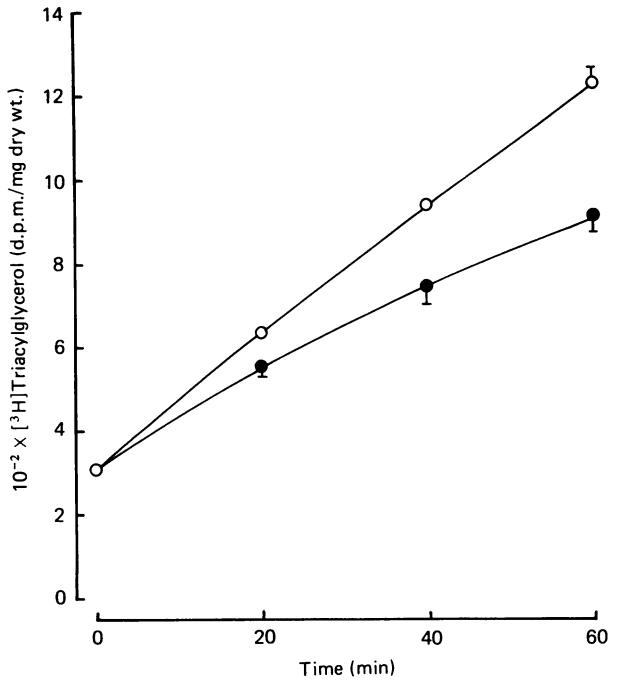

Fig. 2. Effect of (−)adrenaline on secretion of pre-labelled [^{3}H]triacylglycerol from hepatocytes

Hepatocytes were preincubated for 45 min in medium containing 0.5 mM-oleate and 5 mM-[1,2,3-^{3}H]glycerol. Cells were then sedimented, washed and incubated in [^{3}H]-glycerol-free medium in the absence (○) or the presence (●) of 10 μM-(−)adrenaline. The hormone was added at 0 min. [^{3}H]Triacylglycerol content of the medium was measured at the times indicated. Results are means ± S.D. for a single representative experiment.

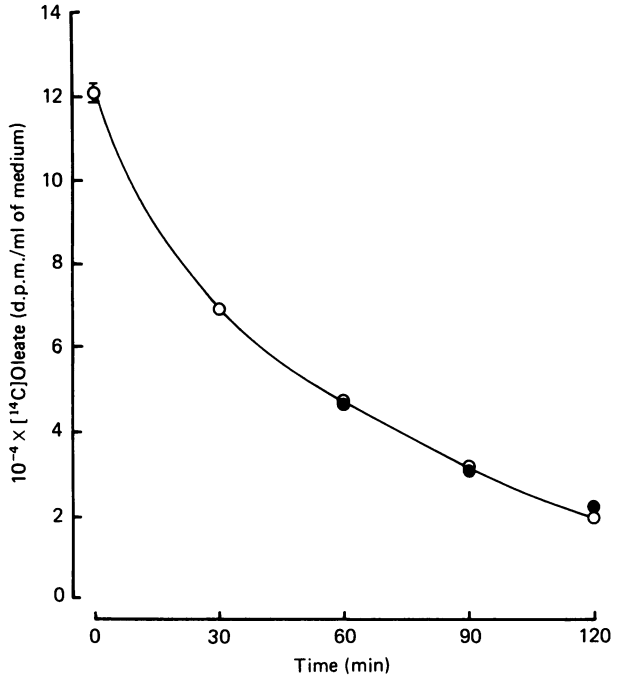

Fig. 3. Effect of (−)adrenaline on the [1-^{14}C]oleate content of the incubation medium

Hepatocytes were incubated in the absence (○) and the presence (●) of 10 μM-(−)adrenaline. When present, (−)adrenaline was added at 30 min. The initial [^{14}C]oleate concentration in the incubation medium was 0.5 mM. Results are means ± S.D. for a single representative experiment.

uptake by hepatocytes. In contrast, the catecholamine did stimulate oxidation of [^{14}C]oleate (Fig. 4). Table 2 shows the effect of adrenaline, alone and with receptor antagonists, on uptake and oxidation of oleate measured between 30 and 90 min of incubation. Oxidation of fatty acid to CO_2 was greater in the presence of adrenaline (Table 2). Stimulation of oleate conversion into CO_2 by adrenaline was abolished by inclusion of prazosin, but not propranolol. Thus, in these cells adrenaline is acting via an α_1-receptor-mediated mechanism to increase oxidation to CO_2. Previous studies have shown α_1-adrenergic stimulation of fatty acid oxidation in rat hepatocytes (Sugden *et al.*, 1980; Kosugi *et al.*, 1983; Oberhaensli *et al.*, 1985; Nomura *et al.*, 1986). In some studies this was associated with an increase in ketogenesis (Kosugi *et al.*, 1983; Oberhaensli *et al.*, 1985), whereas in others rates of ketogenesis were unchanged (Nomura *et al.*, 1986).

DISCUSSION

The data presented in this paper show that stimulation of α_1-adrenergic receptors in isolated rat hepatocytes decreases the secretion of triacylglycerol. This triacylglycerol is present in a form with properties similar to VLDLs. Adrenaline does not affect the re-uptake or degradation of newly secreted triacylglycerol; rather, the catecholamine acts to suppress the rate of secretion of presynthesized glycerolipid from the cells. This implies that adrenaline may decrease the rate of transport of the

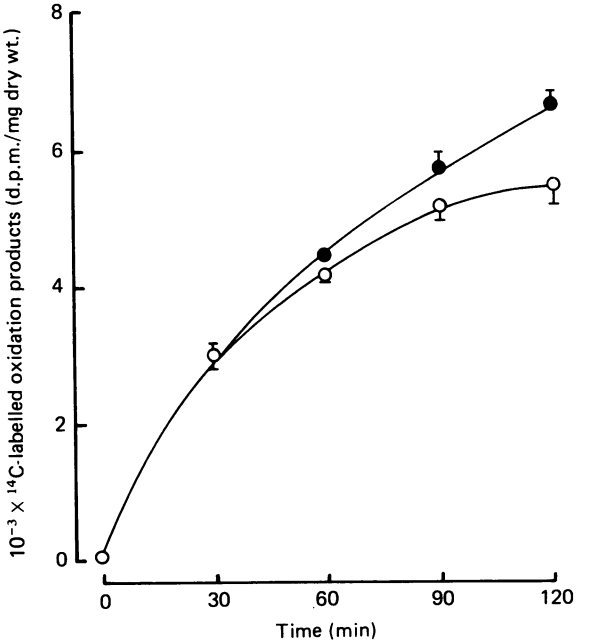

Fig. 4. Effect of (−)adrenaline on [1-^{14}C]oleate oxidation

Hepatocytes were incubated with 0.5 mM-[1-^{14}C]oleate in the absence (○) or presence (●) of 10 μM-(−)adrenaline. When present, (−)adrenaline was added at 30 min. $^{14}CO_2$ and ^{14}C-labelled water-soluble products were measured at the times indicated. Results are means ± S.D. for a single representative experiment.

Table 2. Effect of (−)adrenaline in the absence and the presence of adrenergic antagonists on fatty acid uptake and oxidation by isolated hepatocytes

Hepatocytes were incubated for 90 min in medium containing 0.5 mM-[1-^{14}C]oleate. When present, (−)adrenaline was added at 30 min to a final concentration of 10 μM, and prazosin and propranolol at 1 μM and 10 μM respectively. Rates of uptake and oxidation were measured between 30 and 90 min. Results are expressed as a percentage of the control value, and are means ± S.E.M. for the numbers of preparations shown in parentheses. Absolute values are given in brackets for controls as means ± S.E.M.; rates are expressed as d.p.m./h per mg dry wt. The significance of differences from control values were calculated by using a paired t test, and they are indicated by: $*P < 0.05$; $**P < 0.01$.

Conditions	Value (% of control)			
	Fatty acid uptake	Oxidation products		
		Total	Acid-soluble	CO_2
Control	100 (7) [8468 ± 893]	100 (6) [3443 ± 686]	100 (6) [1969 ± 524]	100 (6) [1413 ± 190]
+ Adrenaline	100 ± 2 (7)	130 ± 5** (6)	129 ± 12 (7)	142 ± 9** (7)
+ Adrenaline + prazosin	103 ± 3 (4)	110 ± 2* (4)	107 ± 5 (4)	116 ± 7 (4)
+ Adrenaline + propranolol	100 ± 3 (4)	127 ± 6* (4)	111 ± 8 (4)	152 ± 13** (4)

newly synthesized particles to the cell surface or inhibit their release. It is also possible that adrenaline affects incorporation of lipid into nascent lipoproteins (Chao *et al.*, 1986). Another catecholamine, noradrenaline, has been shown to inhibit secretion of cholesterol and protein by rat hepatocytes (Edwards *et al.*, 1979). The effect observed in the present study may therefore be a reflection of a general inhibitory action of catecholamines on hepatic secretory mechanisms.

The point in the secretory or release process at which adrenaline exerts its inhibitory effect is not known. Secretion of molecules by cells is an energy-requiring process (Jamieson & Palade, 1968). The lack of effect of the hormone on whole-cell ATP content (control, 9.1 ± 0.4, adrenaline, 8.9 ± 0.5 nmol/mg dry wt.), however, suggests that adrenaline does not inhibit secretion by affecting gross energy status of the cells. Nevertheless, it is possible that the hormone may decrease ATP content at a particular subcellular location. A further potential mechanism for suppressing triacylglycerol secretion is by modulation of intracellular Ca^{2+} concentrations. α_1-Adrenergic stimulation of hepatocytes is associated with a redistribution of intracellular free Ca^{2+} (see Reinhart *et al.*, 1984; Exton, 1985), and this may disrupt local concentrations important in the secretory process.

In addition to the effect of adrenaline on triacylglycerol secretion, the hormone also stimulates oxidation of fatty acid. Particularly, α_1-adrenergic stimulation was associated with an increase in conversion of fatty acid into CO_2 (Table 2). Previous reports have documented the stimulatory effect of α_1-adrenergic action on fatty acid oxidation (see earlier) and flux through the tricarboxylic acid cycle (Taylor *et al.*, 1983; Oberhaensli *et al.*, 1985). The enhancement of oleate conversion into CO_2 observed in the present experiments may therefore be a reflection on increased consumption of acetyl-CoA, derived from elevated rates of oleate oxidation, via the tricarboxylic acid cycle.

The source of the extra oleate consumed on adrenergic stimulation of oxidation in the hepatocytes is unclear. Adrenaline does not increase oleate uptake by the cells (Table 2). The decrease in both cellular [^{14}C]-phospholipid content and secreted [^{14}C]triacylglycerol, together, could account for the increased oleate oxidation. Adrenaline inhibits triacylglycerol output by suppressing the rate of secretion of pre-synthesized lipid (Fig. 3). It may be, therefore, that triacylglycerol accumulating in the secretory pathway of adrenaline-treated cells is subject to lipolysis; the fatty acid released could then contribute to oxidation. Such a mechanism would prevent excessive accumulation of triacylglycerol in adrenaline-stimulated hepatocytes.

Antagonism of the action of adrenaline on triacylglycerol output by prazosin indicates α_1-adrenoceptor involvement. Other reports (Blair *et al.*, 1979; Morgan *et al.*, 1983) have shown that the α-adrenergic receptor is the predominant subtype present on the surface of liver cells from fed, mature, male rats. This does not exclude the possibility that stimulation of hepatic β-adrenoceptors might also affect triacylglycerol secretion. Indeed catecholamine action via β-receptors is associated with an elevation of intracellular cyclic AMP concentration (see Lefkowitz & Caron, 1985), and rates of hepatic triacylglycerol output are decreased in livers treated with dibutyryl cyclic AMP (Heimberg *et al.*, 1969; Klausner *et al.*, 1978; Soler-Argilaga *et al.*, 1978*a*; Kempen, 1980).

The effects of adrenaline observed in the present study may be important in the regulation of hepatic lipoprotein secretion *in vivo*. It is possible, for example, that hepatic lipid metabolism may be modulated by the sympathetic nervous system via adrenergic neurotransmitters. The inhibitory actions of adrenaline on triacylglycerol secretion may also help explain decreases in plasma triacylglycerol content observed in humans (Carruthers & Taggart, 1973; Chait *et al.*, 1979) and animals (Robertson & Smith, 1976) during periods of acute stress. This decrease has been attributed to a suppression of hepatic VLDL secretion and correlated with urinary noradrenaline output (Chait *et al.*, 1979). It is interesting that acute stress is not associated with depressed VLDL secretion in patients exhibiting impaired sympathetic innervation owing to spinal-cord transections (Chait *et al.*, 1979). Again, this suggests involvement of a neural element in the regulation of hepatic triacylglycerol

secretion. In contrast with the acute situation, chronic stress is associated with hypertriacylglycerolaemia (Carlson *et al.*, 1968; Taggart & Carruthers, 1971). The stimulatory effect of glucocorticoids on hepatic lipoprotein secretion (see the Introduction) may be important under these chronic conditions.

The present work demonstrates α_1-adrenergic suppression of triacylglycerol secretion from liver cells. Such regulation may be important in the neural and hormonal co-ordination of hepatic lipoprotein metabolism. In addition, adrenaline may prove a useful tool in delineating important regulatory site(s) in the pathway of VLDL secretion.

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