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SUPPRESSION OF TRIGLYCERIDE SECRETION BY EPINEPHRINE IN ISOLATED RAT HEPATOCYTES

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The effect of epinephrine on triglyceride synthesis and secretion was examined in isolated rat hepatocytes. Epinephrine potently inhibited triglyceride secretion but did not affect cellular triglyceride content or the rate of incorporation of radiolabelled glycerol into cell triglyceride. The inhibitory effect of epinephrine was abolished by inclusion of the α-adrenergic antagonist prazosin but not the β-antagonist propranolol.

There has been a growing interest in recent years in the direct effects of catecholamines on liver metabolism. Attention has been focused especially on the influence of these hormones on glycogenolysis and glucose metabolism (1-5), and it is thought that they have an important role in glucose homeostasis (6). In addition to its central function in carbohydrate metabolism the liver is a major site for the synthesis of triglyceride and its secretion into the blood in the form of very low density lipoproteins. Very little is known, however, about how catecholamines influence hepatic lipid synthesis and secretion.

Previous studies on the direct effects of epinephrine and norepinephrine on hepatic triglyceride metabolism were conducted on the perfused rat liver (7,8). These studies were complicated by the effects of the catecholamines on liver vasculature which necessitated the inclusion of an α-adrenergic antagonist in the

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perfusate. This antagonist would also negate metabolic effects mediated via α-adrenergic receptors.

In the present investigation the effect of epinephrine on triglyceride metabolism was examined in isolated rat hepatocytes. By using this isolated cell system vasoactive effects of the catecholamine are avoided and direct metabolic effects can be determined.

Materials and Methods

Chemicals

[1,2,3-^{3}H]glycerol was from New England Nuclear. Collagenase was from Cooper Biomedical. Prazosin hydrochloride was a gift from Pfizer International and propranolol hydrochloride was from Sigma Chemical Co. The sources of all other chemicals were as previously described (9).

Hepatocyte Isolation

Hepatocytes were isolated from male Holtzman rats (Charles River Crl: CDH (SD)BR) 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 (10) containing 0.035% (w/v) collagenase, 5 mM $CaCl_2$ and 20 mM D glucose. More than 90% of the cells excluded Trypan blue (0.15% in isotonic saline).

Hepatocyte Incubations

Cells were incubated at 37°C (24-30 mg dry wt/flask) in 4 ml of medium (10) containing 2% (w/v) bovine serum albumin, 5 mM [^{3}H]glycerol (1 μCi/ μmole), 0.5 mM oleate and 20 mM D glucose in silicone-treated Erlenmeyer flasks with rubber stoppers. These were gassed for 2 min with O_2:CO_2 (95:5) and shaken at 90 cycles/min. When present hormones and antagonists were added just before the incubation was started. Incubations were terminated by separating cells from medium by centrifugation (600 g x 2 min). Where appropriate, rates of synthesis and secretion were determined between 30 and 90 min.

Lipid Analyses

Lipids were extracted from aliquots of medium and cells according to Folch et al. (11). Water soluble radioactivity was removed by washing once with water and nine times with blank upper phase (11). Triglyceride from the medium was measured fluorometrically (12) and cellular triglyceride as previously described (13). Aliquots of chloroform extracts were dried and lipid associated radioactivity quantified by liquid scintillation counting. Lipid classes were separated by thin layer chromatography (13).

Results

The time course of triglyceride secretion by isolated hepatocytes is shown in Fig. 1. In the presence of 10^{-5} M

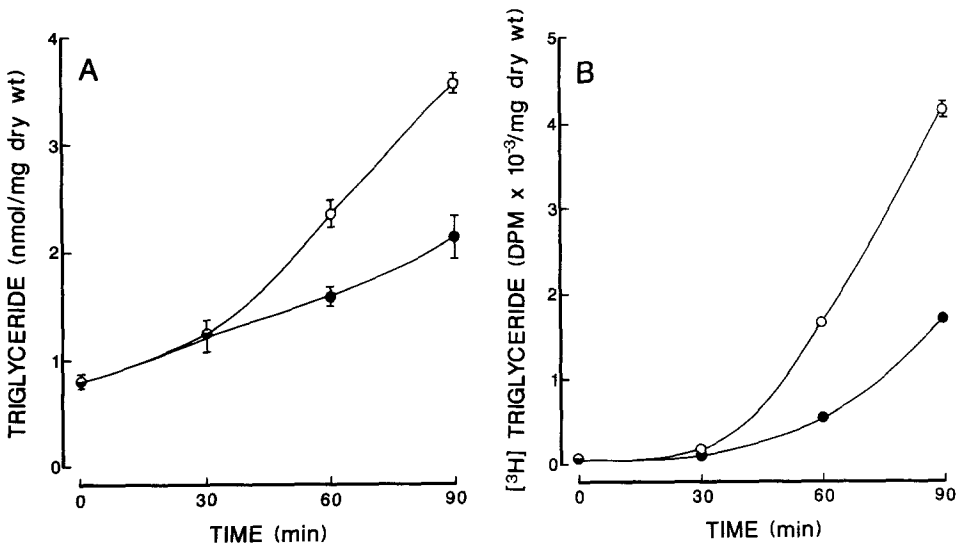

Fig. 1. Time course of triglyceride secretion. Hepatocytes were incubated with [^{3}H]glycerol in the absence (open symbols) or presence (closed symbols) of 10^{-5} M (-)epinephrine as described in Materials and Methods. Triglyceride (1A) and [^{3}H]triglyceride (1B) were determined in the incubation medium at the times indicated. Results are means ± S.D. for a single representative experiment.

(-)epinephrine triglyceride secretion, whether measured directly (Fig. 1A) or radiochemically (Fig. 1B), was markedly diminished.

Figure 2 shows the triglyceride content of the isolated liver cells and the incorporation of [^{3}H]glycerol into cellular triglyceride over a 90 min period. In contrast to its effect on triglyceride secretion, epinephrine had no significant effect on cellular triglyceride content or rate of synthesis from [^{3}H]glycerol.

In further experiments rates of triglyceride secretion and synthesis were determined for hepatocytes incubated alone, with epinephrine or with epinephrine in the presence of the α-adrenergic antagonist prazosin or the β-antagonist propranolol. The results of these studies are shown in Table 1. These data confirm that epinephrine potently inhibits triglyceride secretion. Further, the inhibitory effect of the catecholamine on secretion was blocked by the inclusion of prazosin. In the

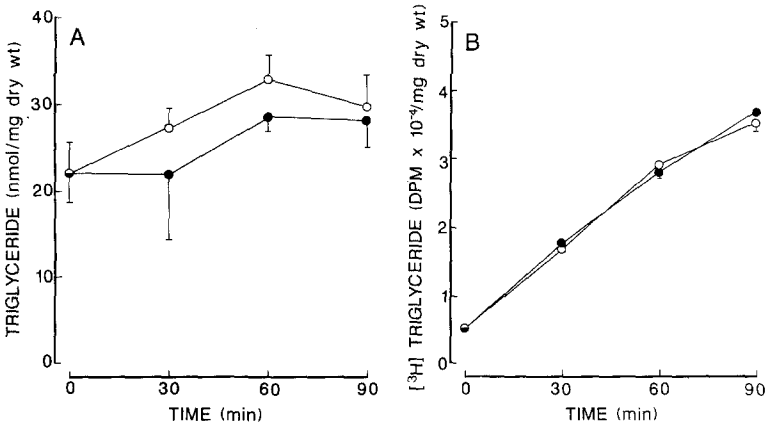

Fig. 2. Cellular triglyceride content (2A) and incorporation of [^{3}H]glycerol into cellular triglyceride (2B). Hepatocytes were incubated with [^{3}H]glycerol in the absence (open symbols) or presence (closed symbols) of 10^{-5} M (-)epinephrine as described in Materials and Methods. Triglyceride content (2A) and [^{3}H] triglyceride (2B) were determined in the liver cells at the times indicated. Results are means ± S.D. for a single representative experiment.

presence of propranolol, however, the hormone still inhibited secretion. Cellular triglyceride content and rates of synthesis were not affected by epinephrine alone or with antagonists.

TABLE 1. Effects of (-)epinephrine in the absence and presence of adrenergic antagonists on triglyceride synthesis and secretion in isolated hepatocytes

		% of Control			
		Medium		Cells	
Conditions		Triglyceride secretion	[^{3}H]triglyceride secretion	Triglyceride content	[^{3}H]triglyceride synthesis
I.	Control	100 (6) (1.9 ± 0.2)	100 (6) (2812 ± 339)	100 (5) (29.3 ± 3.3)	100 (6) (14116 ± 1358)
II.	+Epinephrine	37 ± 6* (6)	44 ± 5* (6)	103 ± 6 (5)	102 ± 6 (6)
III.	+Epinephrine + Prazosin	91 ± 7 (3)	100 ± 3 (3)	96 ± 2 (3)	93 ± 3 (3)
IV.	+Epinephrine + Propranolol	41 ± 7* (3)	51 ± 6* (3)	104 ± 3 (3)	98 ± 7 (3)

Hepatocytes were incubated with [^{3}H]glycerol as described in Materials and Methods. When added, (-)epinephrine was present at 10^{-5} M; prazosin and propranolol at 10^{-6} M and 10^{-5} M, respectively. Results are expressed as percentage of control value and are means ± S.E.M. for the number of preparations shown in parentheses. Absolute values for the controls are given in parentheses in row I as means ± S.E.M. Absolute values for triglyceride secretion and cellular triglyceride contents are expressed as nmole/h per mg dry wt and nmole/mg dry wt, respectively. Absolute values for [^{3}H]triglyceride secretion and [^{3}H]triglyceride synthesis are expressed as dpm/h per mg dry wt. Significant differences from control values are denoted by an asterisk ('t' test, $p < 0.001$).

Discussion

The rates of triglyceride secretion by hepatocytes in the present study compare favorably with rates reported for perfused liver (14) and hepatocytes in culture (15). In these systems, as well as in hepatocytes in suspension (16), triglyceride is secreted predominantly in the form of very low density lipoproteins.

The most notable observation of the present investigation was the marked inhibition of triglyceride secretion by epinephrine. The mechanism whereby epinephrine produces this effect is as yet unclear. It is possible that the catecholamine has an inhibitory effect specifically on the synthesis of triglyceride which is destined for secretion. Epinephrine has been shown to stimulate hepatic ketogenesis *in vitro* via an α-adrenergic mechanism (17). Thus, if the same fatty acid pool were utilized for oxidation and synthesis of secretory triglyceride, a stimulation of the former would divert substrate away from esterification and secretion.

Another possibility is that epinephrine acts to inhibit the assembly of triglyceride into very low density lipoprotein and/or has a negative effect on the secretory process. It is noteworthy in this regard that norepinephrine has been shown to decrease both cholesterol and protein efflux from rat hepatocytes (18).

The observation that inclusion of prazosin, but not propranolol in incubations abolishes the inhibitory actions of epinephrine indicates the hormone is acting via α-adrenergic receptors. α-Adrenoceptor stimulation in rat hepatocytes has been shown to result in an elevation of cytoplasmic free calcium concentrations and 1,2-diacylglycerol (19-21). It is interesting that in some other secretory cell types these two intracellular effectors have been associated with a stimulation of the secretory process (22).

Extrapolating the present observations to the effects of elevated catecholamines on hepatic lipid secretion in vivo is complicated by the actions of these hormones on extrahepatic systems (23) including adipose tissue lipolysis (24) and hemodynamics (25). However, it has been reported that infusion of norepinephrine into anaesthetized rats results in a decrease in hepatic triglyceride output (26). This may well be the result of direct effects of the catecholamine on the liver similar to those reported here for epinephrine.

Acknowledgement

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