Biochem. J. (1981) **198**, 159–166
Printed in Great Britain

Possible role of lipoprotein lipase in the regulation of endogenous triacylglycerols in the rat heart

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(*Received 1 December 1980/Accepted 8 April 1981*)

1. Adrenaline has a biphasic effect on intracellular lipoprotein lipase activity and on endogenous triacylglycerol content in heparin-perfused heart. 2. A high concentration of adrenaline (1 μM in the perfusion buffer) activated endogenous lipoprotein lipase activity and, at the same time, decreased intracellular triacylglycerol stores. 3. In contrast, a low concentration (0.005 μM-adrenaline) inhibited intracellular lipoprotein lipase activity. Under these conditions, cardiac triacylglycerol content was elevated above control values. 4. Perfusing the heart with high and low concentrations of 3-isobutyl-1-methylxanthine elicited a biphasic effect on endogenous lipoprotein lipase activity and triacylglycerol content similar to that seen with adrenaline treatment. 5. The effect of adrenaline on intracellular lipoprotein lipase activity appears to be mediated by cyclic AMP through protein kinase. 6. A possible role for intracellular lipoprotein lipase in the regulation of endogenous triacylglycerol in rat heart is proposed.

In a previous study (Oscai, 1979), suggestive evidence was provided to show that lipoprotein lipase acts as a regulator of intracellular triacylglycerols in the rat heart. In this previous study, glucagon caused marked alterations in the activity of lipoprotein lipase that correlated well with changes in the concentrations of triacylglycerol and non-esterified fatty acids in heart parenchymal cells. The results of the present investigation clearly demonstrate that adrenaline has a biphasic effect on intracellular lipoprotein lipase activity and on endogenous triacylglycerol content similar to that seen with glucagon. In addition, the results show that our intracellular triacylglycerol lipase from heart has characteristics similar to those described for lipoprotein lipase (Korn, 1955*a*,*b*; Robinson, 1970; Borensztajn, 1979). In separate experiments, evidence was provided to show that the effect of hormone on lipoprotein lipase activity is mediated by cyclic AMP, possibly through protein kinase.

Experimental

Treatment of animals

Male rats of a Wistar strain weighing approx. 90g were purchased from Charles River (Wilmington, MA, U.S.A.). Animals were provided access to a diet of Purina chow and water *ad libitum*. They were permitted about 3 weeks to adapt to the living conditions in our animal quarters. At the time of killing, the rats weighed approx. 200g. The animal room was maintained at a temperature between 21 and 23°C and lighted between 07:30h and 19:30h. To control for oscillatory changes in cardiac triacylglycerol lipase activity (Kotlar & Borensztajn, 1977), experiments were started at 08:15h and concluded by 10:15h. After overnight starvation, the rats were anaesthetized with sodium pentobarbital (8–10mg/100g body wt.) and killed by removing the heart.

Heart perfusion

Hearts were isolated and perfused for 2min at a rate of 6ml/min with Krebs–Ringer bicarbonate buffer (pH 7.4) containing 5% (v/v) rat serum and 5 units of heparin/ml to wash lipoprotein lipase, triacylglycerol, and non-esterified fatty acid from the capillary beds. At the end of the 2min perfusion period, the perfusates were free from lipoprotein lipase activity, triacylglycerol and non-esterified fatty acid. The hearts were then perfused for 30min (6ml/min) with Krebs–Ringer bicarbonate buffer containing 5% rat serum and either adrenaline, $N^6O^{2'}$-dibutyryl cyclic AMP or 3-isobutyl-1-methylxanthine. Preliminary results showed that the capillary beds were lipoprotein lipase-free after the 30min perfusion period. Next, the hearts were perfused for 2min at a rate of 6ml/min with Krebs–Ringer bicarbonate buffer containing 5% rat serum to wash adrenaline, dibutyryl cyclic AMP or 3-isobutyl-1-methylxanthine from the capillary beds. Hearts were perfused in a non-recirculatory system

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as described previously (Oscai, 1979). The perfusion medium was continually gassed with O_2/CO_2 (19:1). The perfusion apparatus was housed in a temperature-regulated acrylic cabinet (Medical Research Apparatus, Clearwater, FL, U.S.A.) in which the temperature was maintained at 37°C during the heart perfusions.

Heart homogenate preparation

Hearts were isolated and perfused for 2 min at a rate of 6 ml/min with Krebs–Ringer bicarbonate buffer (pH 7.4) containing 5% rat serum and 5 units of heparin/ml to wash lipoprotein lipase, triacylglycerol and non-esterified fatty acid from the capillary beds. At the end of the 2 min perfusion period, the perfusates were free from lipoprotein lipase activity, triacylglycerol and non-esterified fatty acid. Hearts were then homogenized and used for cyclic AMP, AMP or purified catalytic subunit of protein kinase experiments.

Assay methods

Lipoprotein lipase activity was measured by the assay described by Borensztajn *et al.* (1972). Heparin-perfused hearts were homogenized in 0.025 M/NH_3/HCl buffer (pH 8.1) with a Duall ground-glass grinder (Kontes Glass Co., Evanston, IL, U.S.A.). The tissue concentration of the homogenates was 50 mg/ml. The composition of the assay medium and the procedures for incubation, extraction and measurement of unesterified fatty acids released into the assay medium have been described previously (Borensztajn *et al.*, 1972). Lipoprotein lipase activities are expressed as units ± S.E.M., 1 unit representing 1 μmol of unesterified fatty acids released into the assay medium/h of incubation per g wet wt. of tissue. The enzyme activities were linear with time regardless of tissue treatment.

The hearts were carefully cleaned free of any adherent adipose tissue and connective tissue. Triacylglycerols were extracted from the heart with ethano/diethyl ether (3:1, v/v) by the method of Entenman (1957). The concentration of triacylglycerol was determined as described by Fletcher (1968). Non-esterified fatty acids were measured by the method of Trout *et al.* (1960). The buffer and the tissue concentration of the homogenates for the non-esterified fatty acid assay were the same as those used for the assay of lipoprotein lipase.

Materials

DL-Adrenaline, cyclic AMP, dibutyryl cyclic AMP, AMP and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. 3-Isobutyl-1-methylxanthine was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Protein kinase catalytic subunit from rabbit skeletal muscle was a gift from Dr. S. Whitehouse and Dr. D. A. Walsh (University of California at Davis, CA, U.S.A.) prepared by the method of Beavo *et al.* (1974).

Statistical methods

Results are expressed as means ± S.E.M. Means were compared by Student's *t* test.

Results

Adrenaline experiments

Hearts were perfused with 1 μM-adrenaline. Under these conditions, lipoprotein lipase activity was elevated 2.2-fold, triacylglycerol decreased 62% and non-esterified fatty acid increased 3.5-fold (Table 1). In contrast, perfusing the heart with a low concentration of adrenaline (5 nM) caused a 70% decrease in intracellular lipoprotein lipase activity. At the same time, triacylglycerols were elevated 73% above control values and non-esterified fatty acids were decreased by 80%. Fig. 1(*a*) shows a strong negative correlation between endogenous lipoprotein lipase activity and triacylglycerol content as a result of perfusing the heart with various concentrations of adrenaline. A strong positive correlation was obtained between intracellular lipoprotein lipase activity and cardiac non-esterified fatty acid (Fig. 1*b*).

3-Isobutyl-1-methylxanthine experiments

Table 1 shows that perfusing the heart with 0.7 mg of 3-isobutyl-1-methylxanthine/ml resulted in a 2.1-fold increase in intracellular lipoprotein lipase activity and a corresponding 69% decrease in endogenous triacylglycerol. Non-esterified fatty acids were increased 3.5-fold. On the other hand perfusing the heart with 0.005 mg of 3-isobutyl-1-methylxanthine/ml caused an 85% decrease in intracellular lipoprotein lipase activity. Under these conditions, endogenous triacylglycerols were increased 2.5-fold and cardiac non-esterified fatty acids decreased 70%. Fig. 2(*a*) shows a high negative correlation between intracellular lipoprotein lipase activity and the concentration of triacylglycerol as a result of perfusing the heart with various concentrations of 3-isobutyl-1-methylxanthine. A high positive correlation was obtained between endogenous lipoprotein lipase activity and cardiac non-esterified fatty acid (Fig. 2*b*).

Adrenaline plus 3-isobutyl-1-methylxanthine experiments

In preliminary dose–response experiments, maximal values for lipoprotein lipase activity were attained with concentrations of 1 μM for adrenaline and 0.7 mg/ml for 3-isobutyl-1-methylxanthine (Table 1). When 1 μM-adrenaline and 0.7 mg of 3-isobutyl-1-methylxanthine/ml were included in the perfusion buffer in combination, a new higher

Table 1. *Lipoprotein lipase activity, triacylglycerols and non-esterified fatty acids in the heart after perfusion with adrenaline, 3-isobutyl-1-methylxanthine and dibutyryl cyclic AMP*

Values are means ± S.E.M.; numbers of rats in each group are given in parentheses.

Additions to perfusion medium	Lipoprotein lipase activity (units/g of tissue)	Triacylglycerol (μmol/g of tissue)	Non-esterified fatty acid (μmol/g of tissue)
High concentration			
Adrenaline (1 μM) (8)	162 ± 0.1*	0.68 ± 0.05*	11.85 ± 0.01*
3-Isobutyl-1-methylxanthine (0.7 mg/ml) (5)	155 ± 0.5*	0.55 ± 0.05*	11.92 ± 0.06*
Dibutyryl cyclic AMP (1 mg/ml) (8)	152 ± 1.0*	0.87 ± 0.02*	10.27 ± 0.10*
Control (10)	73 ± 0.2	1.79 ± 0.03	3.40 ± 0.05
Low concentration			
Adrenaline (0.005 μM) (4)	22 ± 0.2*	3.10 ± 0.10*	0.68 ± 0.05*
3-Isobutyl-1-methylxanthine (0.005 mg/ml) (5)	11 ± 0.5*	4.44 ± 0.10*	1.02 ± 0.11*
Dibutyryl cyclic AMP (0.0025 mg/ml) (8)	22 ± 1.0*	3.70 ± 0.13*	0.92 ± 0.10*

* Adrenaline, 3-isobutyl-1-methylxanthine or dibutyryl cyclic AMP versus control, $P < 0.001$.

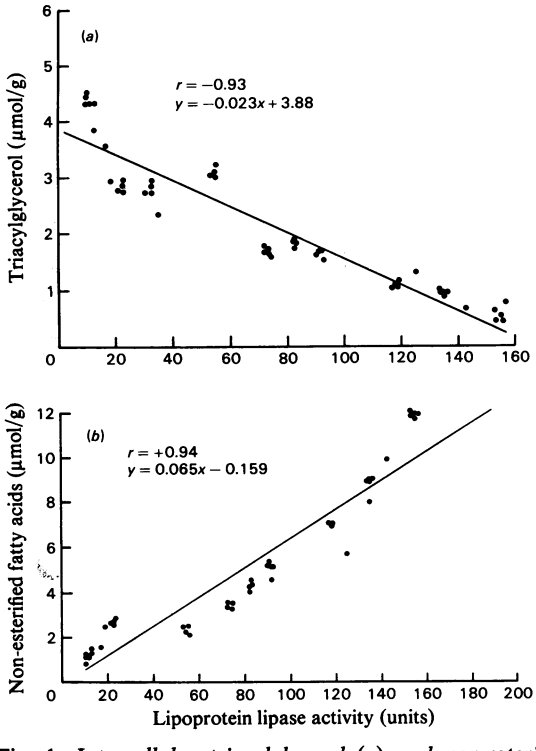

Fig. 1. *Intracellular triacylglycerol (a) and non-esterified fatty acid (b) content plotted as a function of endogenous lipoprotein lipase activity assayed on hearts perfused with various concentrations of adrenaline*

Assays for triacylglycerols, non-esterified fatty acids and lipoprotein lipase activity were carried out as described in the Experimental section.

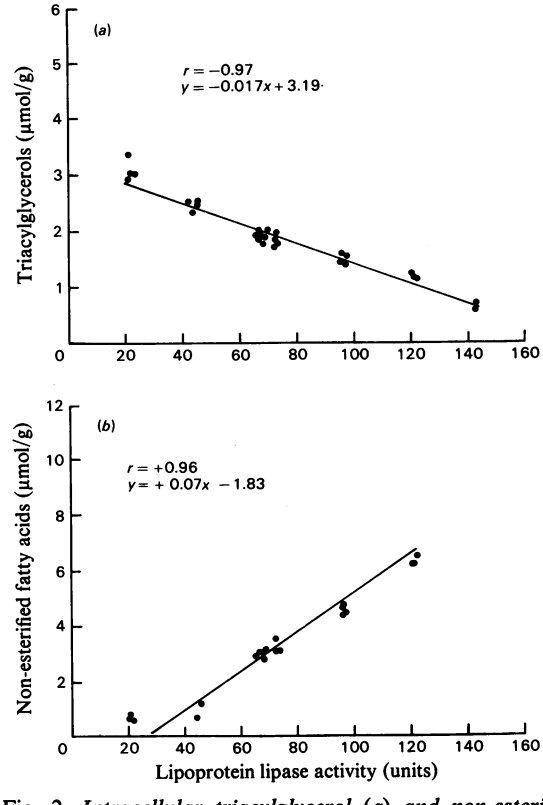

Fig. 2. *Intracellular triacylglycerol (a) and non-esterified fatty acid (b) content plotted as a function of endogenous lipoprotein lipase activity assayed on hearts perfused with various concentrations of 3-isobutyl-1-methylxanthine*

Assays for triacylglycerols, non-esterified fatty acids and lipoprotein lipase activity were carried out as described in the Experimental section.

maximal value of 184 ± 0.4 units/g of tissue was obtained for lipoprotein lipase activity compared with that seen for either agent acting alone ($P < 0.001$). Under these conditions, endogenous triacylglycerol content averaged 0.37 ± 0.04 μmol/g of tissue and non-esterified fatty acids 12.51 ± 0.19 μmol/g of tissue. Perfusion of the heart with a lower concentration of adrenaline (0.5 μM) yielded a submaximal value of 122 ± 0.2 units/g of

tissue for lipoprotein lipase activity. Likewise, perfusion of the heart with a lower concentration of 3-isobutyl-1-methylxanthine (0.1 mg/ml) yielded a submaximal value of 118 ± 0.2 units/g of tissue for lipoprotein lipase activity. In combination, these two agents (0.5 μM-adrenaline plus 0.1 mg of 3-isobutyl-1-methylxanthine/ml) yielded a value of 144 ± 0.5 units/g of tissue, which was higher than that elicited by either agent acting alone ($P < 0.001$).

Dibutyryl cyclic AMP experiments

Table 1 shows that 30 min of perfusion of the heart with a high concentration of dibutyryl cyclic AMP (1 mg/ml) caused an approximate 2-fold increase in lipoprotein lipase activity. Under these experimental conditions, endogenous triacylglycerols were decreased 51% and non-esterified fatty acids were elevated 3-fold. In contrast, perfusion with a low concentration of dibutyryl cylic AMP (2.5 μg/

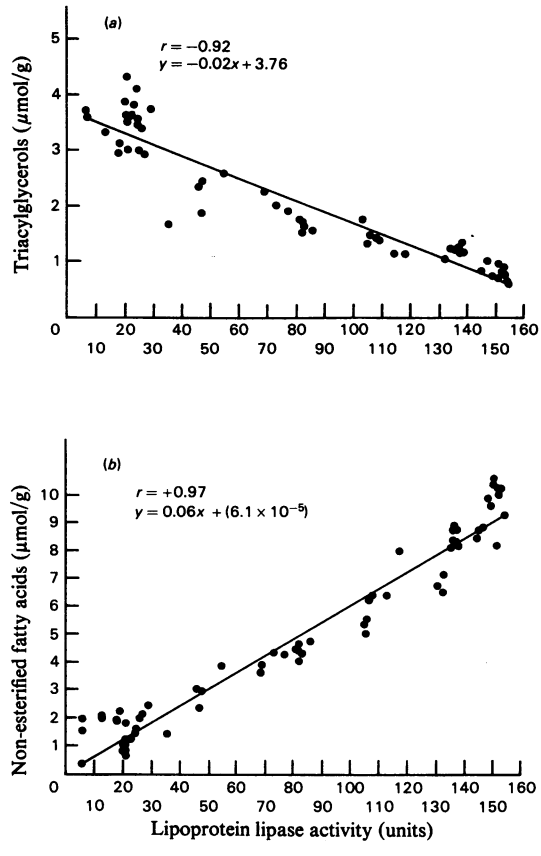

Fig. 3. *Intracellular triacylglycerol (a) and non-esterified fatty acid (b) content plotted as a function of endogenous lipoprotein lipase activity assayed on hearts perfused with various concentrations of dibutyryl cyclic AMP*

Assays for triacylglycerols, non-esterified fatty acids and lipoprotein lipase activity were carried out as described in the Experimental section.

Table 2. *Intracellular lipoprotein lipase activity as a result of adding cyclic AMP or AMP to the homogenizing buffer*

Values are means ± S.E.M.; numbers of rats in each group are given in parentheses.

Nucleotide concentration	Lipoprotein lipase activity (units/g of tissue)
Cyclic AMP	
0.001 μM (4)	22 ± 1
0.01 μM (4)	59 ± 2
0.1 μM (4)	73 ± 1
1.0 μM (4)	88 ± 1
100 μM (4)	108 ± 1
200 μM (4)	114 ± 1
300 μM (4)	141 ± 1
AMP	
0.001 μM (4)	79 ± 1
300 μM (4)	65 ± 1
Control (10)	73 ± 1

Table 3. *Effect of purified catalytic subunit of protein kinase on endogenous lipoprotein lipase activity and non-esterified fatty acids in heart*

Values are means or means ± S.E.M.; numbers of rats in each group are given in parentheses.

Addition of catalytic subunit of protein kinase* (units)†	Lipoprotein lipase activity (units/g of tissue)	Non-esterified fatty acid (μmol/g of tissue)
12.400 (9)	128 ± 1	9.20 ± 0.3
9.900 (2)	124	8.02
1.240 (4)	106 ± 1	6.66 ± 0.2
0.250 (2)	81	5.42
0.030 (2)	77	3.87
0.006 (2)	75	3.61
Control (9)	73 ± 1	3.50 ± 0.1

* Electrophoretically pure catalytic subunit of protein kinase was added to homogenate of heparin-perfused heart and mixed with one pass of the pestle.

† One unit of protein kinase activity = 1 nmol of P_i incorporated into histone/min; 50 μl of protein kinase catalytic subunit in 0.5 mg of bovine serum albumin/ml was added to 2 ml of heart homogenate. No exogenous ATP was added to the homogenate or the assay system.

ml) resulted in a 70% decrease in intracellular lipoprotein lipase activity (Table 1). At the same time, triacylglycerol content was increased 2.1-fold and non-esterified fatty acid decreased 73% (Table 1).

The relationship between lipoprotein lipase activity and triacylglycerol content of the myocardium as a result of perfusing the heart with various concentrations of dibutyryl cyclic AMP was determined. Fig. 3(*a*) shows a high negative correlation between enzyme activity and triacylglycerol concentration. In contrast, a high positive correlation was obtained between enzyme activity and non-esterified fatty acid content of the heart (Fig. 3*b*).

Cyclic AMP and AMP experiments

Table 2 shows that a low concentration of cyclic AMP (1 nM) in the homogenization buffer resulted in a 70% decrease in lipoprotein lipase activity ($P < 0.001$). On the other hand, a high concentration of cyclic AMP (300 μM) in the homogenizing medium caused a 93% increase in cardiac lipoprotein lipase activity ($P < 0.001$; Table 2). As shown in Table 2, a small but statistically significant increase occurred in lipoprotein lipase activity (8%; $P < 0.001$) with a low concentration of AMP in the homogenization buffer and, conversely, a small decrease in enzyme activity (11%; $P < 0.001$) with a high concentration of AMP in the homogenizing buffer.

Purified catalytic subunit of protein kinase experiments

Table 3 gives the levels of activity of lipoprotein lipase and the concentrations of non-esterified fatty acid as a result of adding various amounts of purified protein kinase catalytic subunit to heart homogenate. Lipoprotein lipase activity increased 75% as a result of adding 12.4 units of protein kinase catalytic subunit to heart homogenate. Correspondingly, non-esterified fatty acid content increased 2.6-fold. The addition of higher amounts of protein kinase catalytic subunit to homogenates did not elevate lipoprotein lipase activity above the highest level shown in Table 3 (results not shown). The addition of 0.006 unit of protein kinase catalytic subunit to heart homogenate had no effect on intracellular lipoprotein lipase activity (Table 3). The addition of lesser amounts of protein kinase catalytic subunit to heart homogenate did not lower lipoprotein lipase activity below that seen in hearts from control rats.

Characterization of lipoprotein lipase activity

Table 4 shows the effects of serum and 1 M-NaCl on the lipolytic activities of the heart when assayed using cyclic AMP, protein kinase, dibutyryl cyclic AMP, adrenaline and 3-isobutyl-1-methylxanthine as stimulating agents. In the absence of serum, the inhibition varied from 68% to 92%. In the stimulated condition, the inhibition in the absence of serum was consistently between 110 and 115 units. The addition of 1 M-NaCl to the assay completely inhibited lipoprotein lipase activity regardless of the conditions (Table 4). Figs. 4–6 show the effects of pH, heparin and protamine sulphate on the lipolytic activities of the heart when assayed using the same stimulating agents outlined above for serum and 1 M-NaCl. The results show that maximum levels of lipoprotein lipase activity were reached at pH 8.1 (Fig. 4) and when the concentration of heparin was 14 units/ml (Fig. 5). Fig. 6 shows that protamine sulphate (1 mg/ml) completely inhibited the lipolytic activities of the heart regardless of the experimental conditions.

Table 4. *The effects of serum and 1 M-NaCl on intracellular lipoprotein lipase activity in heart*
Values are means ± S.E.M.; numbers of rats in each group are given in parentheses.

Condition	Stimulated lipoprotein lipase activity		Inhibition in the absence of serum (%)	Inhibition in the absence of serum (units)	Inhibition in the presence of 1 M-NaCl (%)
	+Serum	−Serum			
Homogenization					
Cyclic AMP* (8)	140 ± 1	28 ± 1	80	112	100
Protein kinase† (4)	125 ± 1	10 ± 2	92	115	100
Perfusion					
Dibutyryl cyclic AMP‡ (8)	154 ± 1	39 ± 1	74	115	100
Adrenaline§ (8)	162 ± 1	52 ± 1	68	110	100
3-Isobutyl-1-methylxanthine‖ (8)	154 ± 1	42 ± 1	73	112	100
Control (16)	73 ± 1	15 ± 1	80	58	100

* Homogenizing buffer contained 300 μM-cyclic AMP.
† 12.4 units of catalytic subunit of protein kinase was added to heart homogenate and mixed with one pass of the pestle.
‡ Hearts were perfused with 1 mg of dibutyryl cyclic AMP/ml.
§ Hearts were perfused with 1 μM-adrenaline.
‖ Hearts were perfused with 0.7 mg of 3-isobutyl-1-methylxanthine/ml.

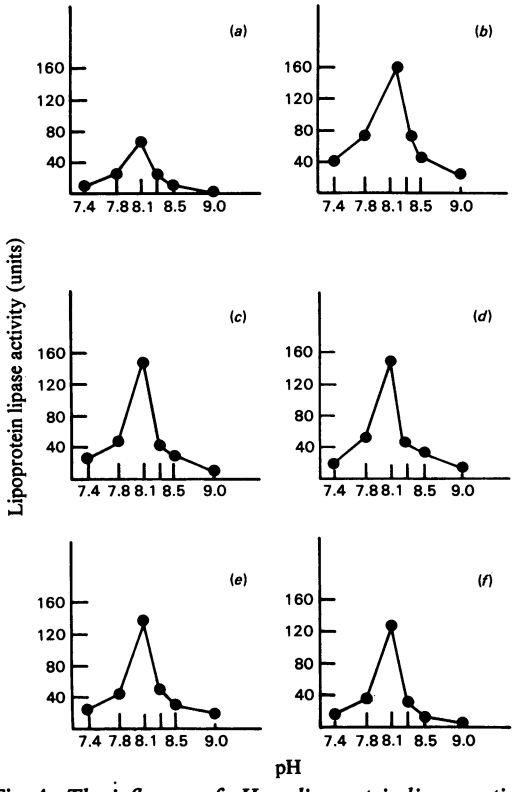

Fig. 4. *The influence of pH on lipoprotein lipase activity measured in homogenates of heparin-perfused rat heart*

The heart received one of the following treatments: non-treated control (*a*); perfusion with 1 μM-adrenaline (*b*); perfusion with 0.7 mg of 3-isobutyl-1-methylxanthine/ml (*c*); perfusion with 1 mg of dibutyryl cyclic AMP/ml (*d*); homogenization in buffer containing 300 μM-cyclic AMP (*e*); and addition of 12.4 units of protein kinase catalytic subunit (50 μl in 0.5 mg of bovine serum albumin/ml) to 2 ml of homogenate (*f*). Homogenates were prepared and assays were performed at the pH indicated. Each point represents the mean for five hearts in (*a*) and two hearts in (*b*)–(*f*).

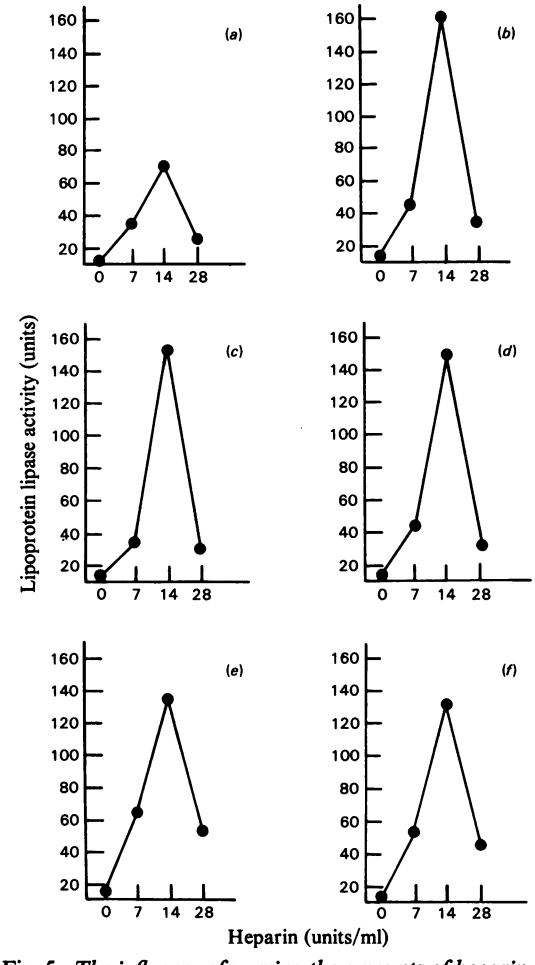

Fig. 5. *The influence of varying the amounts of heparin in the assay on lipoprotein lipase activity measured in homogenates of heparin-perfused rat heart*

The heart received one of the following treatments: non-treated control (*a*); perfusion with 1 μM-adrenaline (*b*); perfusion with 0.7 mg of 3-isobutyl-1-methylxanthine/ml (*c*); perfusion with 1 mg of dibutyryl cyclic AMP/ml (*d*); homogenization with buffer containing 300 μM-cyclic AMP (*e*); and addition of 12.4 units of protein kinase catalytic subunit (50 μl in 0.5 mg of bovine serum albumin/ml) to 2 ml of homogenate (*f*). Each point represents the mean for three hearts in (*a*) and two hearts in (*b*)–(*f*).

Discussion

Adrenaline has been shown to stimulate non-esterified fatty acid and glycerol release from the isolated perfused rat heart (Williamson, 1964; Challoner & Steinberg, 1965, 1966; Kreisberg, 1966). More recently, it was possible to demonstrate a decrease in the concentration of triacylglycerols as a result of perfusing the heart with catecholamines (Gartner & Vahouny, 1972; Crass, 1973; Crass *et al.*, 1975). The lipolytic effects of catecholamines and glucagon in heart appear to be mediated by cyclic AMP (Gartner & Vahouny, 1972; Crass, 1973; Lech *et al.*, 1977). However, although the 'hormone-sensitive lipase' from adipose tissue has been partially purified and has been shown to be activated by cyclic AMP, a direct demonstration of the control of a cardiac lipase by cyclic AMP has not appeared (Lech *et al.*, 1977). Of considerable interest, therefore, are the results of the present study showing that perfusion of the heart with a high concentration of adrenaline caused an increase in the activity of intracellular lipoprotein lipase and, at the same time, a decrease in the concentration of endogenous triacylglycerol.

Cyclic AMP phosphodiesterase is a known

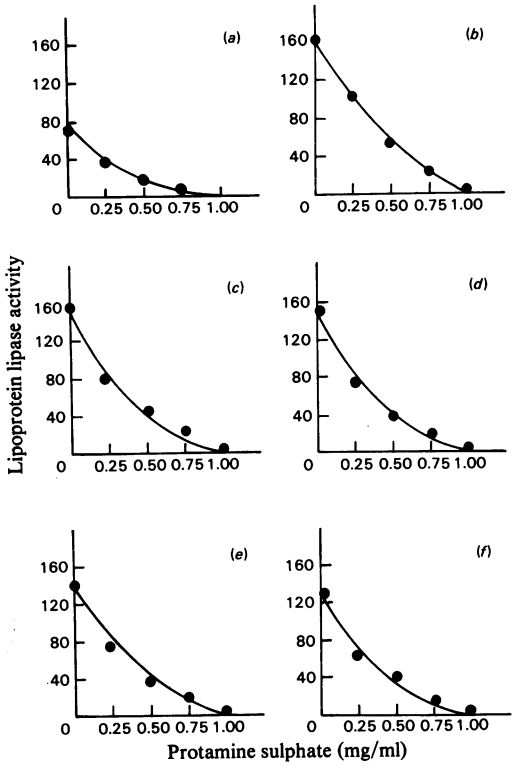

Fig. 6. *The influence of varying the amounts of protamine sulphate on lipoprotein lipase activity measured in homogenates of heparin-perfused rat heart*

The heart received one of the following treatments: non-treated control (*a*); perfusion with 1 μM-adrenaline (*b*); perfusion with 0.7 mg of 3-isobutyl-1-methylxanthine/ml (*c*); perfusion with 1 mg of dibutyryl cyclic AMP/ml (*d*); homogenization with buffer containing 300 μM-cyclic AMP (*e*); and addition of 12.4 units of protein kinase catalytic subunit (50 μl in 0.5 mg of bovine serum albumin/ml) to 2 ml of homogenate (*f*). Each point represents the mean for five hearts in (*a*) and two hearts in (*b*)–(*f*).

inhibitor of cyclic AMP. In turn, Beavo *et al.* (1970) have reported that phosphodiesterase is inhibited by 3-isobutyl-1-methylxanthine. Thus an inhibition of phosphodiesterase activity by 3-isobutyl-1-methylxanthine would favour the accumulation of cyclic AMP. As a result, 3-isobutyl-1-methylxanthine was used as an additional probe to examine the role of lipoprotein lipase as a regulator of intracellular triacylglycerol in rat heart. The data obtained indicate that 3-isobutyl-1-methylxanthine exerts a biphasic effect on lipoprotein lipase activity and triacylglycerol content similar to that seen with adrenaline. Thus activation of lipoprotein lipase activity appears to promote triacylglycerol hydrolysis and, conversely, enzyme inhibition is accompanied by triacylglycerol accumulation above control values in the myocardium. These results are similar to those obtained in a previous study *in vivo* on cardiac lipoprotein lipase activity and triacylglycerol content in glucagon-treated rats (Oscai, 1979). A common denominator with respect to adrenaline, glucagon and 3-isobutyl-1-methylxanthine is that the intracellular effects of these agents are thought to be mediated by cyclic AMP. In keeping with this is the finding that perfusion of the heart with dibutyryl cyclic AMP produced a biphasic effect on intracellular lipoprotein lipase activity and on endogenous triacylglycerol content similar to that seen with adrenaline, glucagon and 3-isobutyl-1-methylxanthine. In the next series of experiments, heparin-perfused hearts were homogenized with buffer containing cyclic AMP or AMP. The results reveal that the activation and inhibition of lipoprotein lipase activity by cyclic AMP appears to be specific to cyclic AMP since AMP has no effect on enzyme activity.

A biphasic phenomenon in lipid metabolism has been described in humans. Eaton (1977) reported that a single intravenous injection of a high dose of glucagon (5 μg/kg body wt.) caused triacylglycerol hydrolysis in circulation of hypertriglyceridaemic individuals, whereas a low dose of glucagon (2 μg/kg body wt.) caused triacylglycerol accumulation above control. This shift in lipid metabolism from hydrolysis to synthesis as a result of decreasing the dose of glucagon has been called the 'glucagon-shift' (Eaton, 1977).

Sutherland's group proposed that in cyclic AMP-regulated processes, when a hormone that induces cyclic AMP is administered in combination with a phosphodiesterase inhibitor, it should be possible to demonstrate an additive physiological effect (Robison *et al.*, 1971). Guided by such a criterion, we measured intracellular lipoprotein lipase activity in hearts of rats perfused with a combination of adrenaline and 3-isobutyl-1-methylxanthine. In combination, these two agents yielded higher values than could be elicited by either agent acting alone.

The first step in cyclic AMP action is generally thought to be the activation of cyclic AMP-dependent protein kinase. Therefore, in the next series of experiments an attempt was made to determine if the increase in lipoprotein lipase activity observed with cyclic AMP treatment could be mimicked by the addition of purified catalytic subunit of protein kinase to whole homogenate of heparin-perfused heart. The finding that protein kinase catalytic subunit resulted in an increase in intracellular lipoprotein lipase activity provides evidence that the effect of cyclic AMP on lipoprotein lipase is protein kinase-mediated and not due to an allosteric binding of cyclic AMP to enzyme protein. Whether the change in lipoprotein lipase activity is caused by phosphoprotein formation remains to be elucidated.

The results of the present study raise some interesting questions and lay the groundwork for more specific studies of the biochemical processes involved. First, the addition of purified catalytic subunit of protein kinase to heart homogenate clearly stimulated lipoprotein lipase activity. Protein kinase catalyses the transfer of the γ-phosphate group from ATP to serine and/or threonine residues on specific acceptor proteins. Thus protein kinase-catalysed phosphorylation has an ATP requirement. Yet, in the present study, cyclic AMP acting through protein kinase appeared to stimulate endogenous lipoprotein lipase activity resulting in cardiac triacylglycerol hydrolysis but in the absence of exogenous ATP in our assay system. At the present time, we do not have an explanation for the lack of an ATP requirement in the activation of lipoprotein lipase. However, we have not ruled out the possibility that protein kinase might be using endogenous ATP. Secondly, the addition of purified catalytic subunit of protein kinase to heart homogenate activated lipoprotein lipase activity at high concentrations but did not inhibit enzyme activity at low concentrations. Although detailed evidence is lacking, it appears that protein kinase is involved in the cyclic AMP stimulation of intracellular lipoprotein lipase activity, but is not involved in the cyclic AMP-related enzyme inhibition.

Lukens & Borensztajn (1978) provide direct evidence that apoprotein C-II promotes the hydrolysis of chylomicron triacylglycerols by endothelium-bound lipoprotein lipase. At the present time, we cannot provide evidence for an intracellular activator of lipoprotein lipase similar to apoprotein C-II for lipoprotein lipase in the capillary beds. Although even the existence of an intracellular activator remains uncertain, it is clear from our results that our intracellular triacylglycerol lipase possesses many of the characteristics described for lipoprotein lipase. The common properties include alkaline pH optimum, serum requirement, activation by heparin, inhibition by high salt concentrations and inhibition by protamine sulphate (Borensztajn, 1979).

Finally, it should be pointed out that Hahn (1943) first stimulated interest in a heparin-induced lipaemia clearing system. About 25 years ago, Korn (1955*a*,*b*) partially characterized a heparin-activated clearing factor and termed it 'lipoprotein lipase'. In the intervening years, the concept has developed that lipoprotein lipase functions exclusively as a clearing-factor lipase responsible for the hydrolysis of triacylglycerols from circulating chylomicrons and very-low-density lipoproteins (Robinson, 1970; Borensztajn, 1979). It is attractive to think that lipoprotein lipase functions within the cell as a regulator of intracellular triacylglycerols in the rat myocardium. The evidence seems reasonably good that the actions of adrenaline and glucagon (Oscai, 1979) on intracellular lipoprotein lipase activity are mediated by cyclic AMP through protein kinase. Since it was possible to demonstrate hormone sensitivity and because lipoproteins do not exist in heart parenchymal cells, it may be more appropriate in the future to call this intracellular enzyme type L hormone-sensitive lipase.

This work was supported by the United States Public Health Service Research Grants AM-17357, K04 AM-00216 and HD-10987.

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