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PROTEIN KINASE ACTIVATION OF HEPARIN-RELEASABLE LIPOPROTEIN LIPASE IN RAT HEART

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SUMMARY: An attempt was made to activate the capillary-bound fraction of lipoprotein lipase (LPL) with cAMP-dependent protein kinase catalytic subunit (PKC). Following a 30s washout period, hearts were perfused for 1 min with buffer containing heparin. Medium was collected during the second 30s of heparin perfusion. Addition of PKC+Mg-ATP to this capillary bed perfusate increased LPL activity from 6.84 ± 0.72 nmol/ml/min to 13.76 ± 1.12 nmol/ml/min ($P<0.001$). A similar 2-fold increase in activity was observed when results were expressed on a mg protein basis. Removal of serum from, or addition of 1.0M NaCl to, the assay system inhibited PKC-stimulated LPL activity approximately 85%. These results indicate that capillary alkaline LPL can be activated by PKC assayed under experimental conditions free of other TG lipases. Moreover, these findings suggest that the intracellular fraction of LPL can be activated by cAMP and that this activation is mediated through protein phosphorylation by cAMP-dependent protein kinase.

In the search for a hormone-sensitive triglyceride (TG) hydrolase in cardiac muscle, activities of an acid, a neutral, and an alkaline lipase have been observed. It remains controversial, however, as to which lipase(s) is responsible for lipolysis of intracellular TG in the heart. Previously, data have been obtained to show that the intracellular fraction of lipoprotein lipase (LPL) (alkaline TG lipase) may function in this role. In this earlier work, glucagon (1), epinephrine, cAMP, cAMP-dependent protein kinase, and 3-isobutyl, 1-methylxanthine (2-4) have been shown to activate intracellular LPL and decrease TG stores in rat heart. One criticism of the work on intracellular LPL is that hormone stimulation of this enzyme may, in fact, be an activation of the neutral TG lipase present in crude heart extracts (5). Currently, it is thought that LPL is synthesized within the tissue cell and, thereafter, designated for secretion and transport to the capillary beds via the microtubular system (6). In the capillary beds, LPL is free from both

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the acid and neutral TG lipases. Therefore, in the present study the endothelium-bound enzyme was used as a tool to demonstrate that LPL can be activated in a system free of other TG lipases and to provide evidence that the mechanism of activation may be regulated by phosphorylation mediated by protein kinase.

MATERIALS AND METHODS

Animal care. Male rats of the Wistar strain (Charles River, Wilmington,MA.), weighing approximately 300g, were used in these experiments. They were provided unrestricted access to a diet of Purina rat chow and water. The animal room was maintained at a temperature between 21 and 23 °C. and lighted between 7:00 A. M. and 7:00 P. M. Rats were housed under these conditions for at least 3 wk prior to experimentation to minimize data variability. Rats were used for experimentation between 7:00 and 8:00 A. M. after an overnight fast.

Enzyme preparation. To obtain capillary-bound LPL, rats were anesthetized with sodium pentobarbital (8-10 mg/100 g body wt). Hearts from two rats were isolated and each perfused for 30s with Krebs bicarbonate buffer (pH 7.4) at a flow rate of 6 ml/min to wash blood from the coronary circulation. Next, the hearts were perfused for 60s with buffer containing 5 units of heparin/ml. Perfusates collected during the last 30s of heparin perfusion for the two rats were pooled and used as the enzyme source. The pooled perfusates were immediately assayed for LPL activity under four conditions: enzyme alone, enzyme plus Mg-ATP(10 mM - 3 mM, respectively), enzyme plus protein kinase catalytic subunit, or enzyme plus Mg-ATP and protein kinase catalytic subunit.

Assays. LPL activity was measured by the method of Nilsson-Ehle and Schotz (7). The final concentration of trioleate was 5.66 mM at a specific activity of 2.9 cpm/pmol. Released free fatty acids (FFA) were extracted by the method of Belfrage and Vaughan (8). The protein content of the perfusate was determined by the method of Lowry et al. (9). All enzyme activities were linear with respect to the amount of perfusate protein in the assay and incubation time.

Materials. Tri-[9,10-^{3}H(N)]oleoylglycerol (19.9 Ci/mmol) was obtained from the New England Nuclear Corp. Protein kinase catalytic subunit, purified to homogeneity by the method of Beavo et al. (10) was a gift from Dr. D. A. Walsh (University of California at Davis, CA).

RESULTS

Table 1 shows that perfusate LPL activity was not changed from basal levels as a result of adding either PKC or Mg-ATP alone. However, the addition of purified cAMP-dependent protein kinase catalytic subunit (PKC) and Mg-ATP, in combination, increased the lipolytic activity in perfusates of heparin-perfused hearts from a control level of 45.25 ± 9.34 to 90.83 ± 17.22 nmol FFA released/min/mg protein (Table 1). The two-fold increase in lipase

TABLE 1

Effect of cyclic AMP-dependent protein kinase catalytic subunit (PKC) and Mg- ATP on lipoprotein lipase activity in perfusates from heparin-perfused rat hearts

Additions	Lipoprotein lipase activity	
	nmol FFA/ml/min	nmol FFA/mg protein/min
None	6.84 ± 0.72	45.25 ± 9.34
Mg-ATP[a]	5.99 ± 0.31	40.05 ± 7.08
PKC	7.14 ± 0.61	47.16 ± 9.29
Mg-ATP + PKC[b]	13.76 ± 1.12[c]	90.83 ± 17.22[d]

Values are Means ± SEM, N=6.
[a] 10 mM Mg++, 3 mM ATP
[b] 0.81 μg of PKC, with an activity of 2.0 μmol of phosphate incorporated into mixed histone/mg/min, was added to perfusate containing LPL and Mg-ATP in a final volume of 130 μl and assayed immediately for lipolytic activity.
[c] Significantly greater than all other mean activities expressed as nmol/ml/min ($P<0.001$).
[d] Significantly greater than all other mean activities expressed as nmol/mg protein/min ($P<0.05$).

activity was evident regardless of whether data were expressed on a mg protein basis or as a function of perfusate volume.

To characterize the lipolytic activity measured in heart perfusates, assays were also performed in the absence of serum or in the presence of 1.0 M NaCl. PKC-activated lipolytic activity was reduced approximately 85% when serum was excluded or NaCl was added to the assay system. These data are consistent with the hypothesis that we were activating the endothelial-bound fraction of lipoprotein lipase.

DISCUSSION

Previously, it was possible to activate intracellular LPL in hearts of intact rats injected with glucagon (1) and in heparin-perfused hearts treated with epinephrine, cAMP, cAMP-dependent protein kinase, and 3-isobutyl, 1-methylxanthine (2-4). However, because of the abundance of LPL in heart tissue, it is possible that the increase seen in intracellular LPL activity

might have masked the activation of a neutral TG lipase (hormone-sensitive lipase). Further, it has been suggested that since LPL is a secretory enzyme and lipolysis of endogenous TG by intracellular LPL has not been demonstrated, it is more likely that the increased lipolysis attributed to intracellular LPL was due to the cAMP activation of the neutral TG lipase reported by Goldberg and Khoo (5). In contrast to this notion, the results of the present study indicate that capillary bed LPL can be activated by protein kinase assayed under experimental conditions free of the acid and/or neutral TG lipases. In addition, these results suggest that the capillary bed LPL activated by cAMP-dependent protein kinase in the present study is the same lipase activated in the intracellular fraction of rat heart by glucagon, epinephrine, cAMP, cAMP-dependent protein kinase, and 3-isobutyl, 1-methylxanthine, reported previously (1-4).

Although the presence of a neutral TG lipase in rat heart has been reported, attempts to activate this lipase with cAMP-dependent protein kinase have not been successful (11,12). Moreover, the 39% increase in a neutral TG lipase activity reported by Goldberg and Khoo (5) may not represent a true activation. For example, these authors (5) first lowered the basal activity of a cardiac TG lipase from about 15 nmol FFA/mg protein/h (heparin-Sepharose treated S_{40} unretained fraction [Fig. 1B, see Ref. 5]) to 2.3 ± 0.1 nmol FFA/mg protein/h by preincubation with Mg^{++} (Table 1, Ref. 5). Addition of cAMP+ATP to the preparation with reduced enzyme activity increased TG hydrolase activity 39% to 3.2 ± 0.2 nmol FFA/mg protein/h. However, the highest cAMP-stimulated activity reported by Goldberg and Khoo was still 79% below basal enzyme activity levels.

Finally, the identification of a hormone-sensitive TG lipase in heart remains controversial. As the search continues, it is necessary to isolate the various intracellular TG lipases (i.e., acid, neutral, and alkaline) and to determine their mechanisms of control. In this context, the results of the present study provide suggestive evidence that the activity of the alkaline lipase from rat heart can be regulated by phosphorylation mediated through cAMP-dependent protein kinase.

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