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Regulation of Rat Heart Lipoprotein Lipase Activity During Cold Exposure[1] (38212)

P. KEIG AND J. BORENSZTAJN
(Introduced by R. W. Wissler)
Department of Pathology, Pritzker School of Medicine, University of Chicago, Chicago, Illinois 60637

Plasma triglyceride fatty acids (TGFA) represent an important source of substrate for oxidation by the heart and skeletal muscle (1–3). In these, as well as in most extra-hepatic tissues, the uptake of TGFA from the plasma is dependent on the enzyme lipoprotein lipase (LPL) (4). In the heart the activity of this enzyme varies according to the physiological state of the animal. Postprandially, when plasma TGFA are not being preferentially oxidized by the myocardium, LPL activity in this tissue is low (1, 5, 6). Conversely, during periods of caloric deficit (e.g., starvation) or increased caloric demands (e.g., exposure to cold temperatures), when oxidation of fatty acids by the myocardium is substantial, LPL activity in this tissue is markedly increased (1, 5, 8).

Little is known regarding the factors which regulate myocardial LPL activity in different physiological conditions. Previous work from this laboratory has shown that the fluctuations in rat heart LPL activity with starvation and feeding may be regulated by the levels of glucagon in the plasma (9). However, glucagon may not be involved in this regulatory process in other physiological situations. For example, the marked increase in LPL activity which is observed in the heart of starved rats exposed to cold temperatures (8) cannot be induced by injecting glucagon into these animals at room temperature (9).

Several other hormones have been implicated in the regulation of myocardial LPL activity (4). Catecholamines and thyroid hormones, for example, have been reported (7, 10, 11) to cause increases in heart LPL activity, although other studies (12, 13) have failed to confirm some of these findings. Since exposure of normal rats to cold temperatures is accompanied by a marked rise in the plasma levels of adrenal and thyroid hormones (14–18), we decided to investigate whether the increase in heart LPL activity induced by cold exposure is dependent on the increased levels of these hormones in circulation. In the present study we compared the LPL activities in the hearts of normal, thyroidectomized and adrenalectomized rats exposed to 4° or maintained at room temperature.

Methods. Male Sprague–Dawley rats (110–160 g) were used in this study. Thyroidectomized, adrenalectomized, adrenal-demedullated and sham operated rats were purchased from Hormone Assay Laboratories, Chicago, Illinois. All animals were operated on 6–8 days before being sacrificed and had free access to food and water with the exception of the adrenalectomized rats which were maintained on a 1% sodium chloride solution. At 6 pm of the day preceding the experiments the animals were deprived of food but not water or salt solution. Twelve hr later some animals were transferred to individual plastic cages and put in a lighted cold room maintained at 4° for 3 hr. The animals were killed by exanguination through the abdominal aorta while under ether anesthesia. LPL activity

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in the tissues was determined as previously described (5, 6) except that the adipose tissue LPL activity was measured in fresh tissue homogenized in 0.025*N* NH_3–NH_4Cl buffer using a Duall ground glass tissue grinder (Kontes Glass Co., Chicago).

The enzyme activities are expressed in units ± standard deviation of the mean, 1 unit representing 1 μmole free fatty acids released per hr incubation. The significance of the difference between means was analyzed using the Student *t* test. Crystalline porcine insulin was a gift from Dr. Ronald Chance of Eli Lilly and Company, Indianapolis.

Results. In Table I are shown the results obtained when sham operated thyroidectomized and adrenalectomized rats were exposed to 4° for 3 hr. The results are expressed as LPL units per g wet wt and LPL units per heart since in one group of animals (thyroidectomized) the average weights of the hearts (350 ± 28, $n = 10$) was significantly lower than those of the controls (426 ± 32, $n = 20$) ($P < 0.001$).

Exposure of sham operated starved rats to cold temperature induced a significant increment in the heart LPL activity. Such increase in LPL activity was also observed in skeletal muscle. In acetone-ether powder preparations of diaphragms from the sham operated animals maintained at room temperature and exposed to 4° for 3 hr the LPL activities were, respectively, 36 ± 12.5 and 60 ± 12 LPL units/g wet wt ($n = 10$, $P < 0.001$). In the thyroidectomized and in the adrenalectomized rats exposed to 4°, the heart LPL activities were also significantly higher than those of the animals maintained at room temperature. It is noteworthy that, due to differences in weight, the LPL activity in the hearts of thyroidectomized rats was comparable to that of the sham operated rats only when expressed as LPL units per heart. The hearts of the adrenalectomized rats, on the other hand, in spite of having weights comparable to those of the sham operated animals, had LPL activities about 50% lower than that of the controls when expressed as LPL units per heart or LPL units per g wet wt. In order to investigate whether this reduced LPL activity in the hearts of adrenalectomized rats was due to the deficiency of adrenal-medullary or cortical hormones, adrenal-demedullated rats were exposed to 4° and their heart LPL activities measured. As shown in Table I the results obtained on these animals were comparable to those obtained on the adrenalectomized rats.

Radomski and Orme (7) reported that the high levels of myocardial LPL activity induced by cold exposure could be partially reversed by injecting the animals with insulin. We have recently reported (6), however, that the LPL activity in the hearts of rats maintained at room temperature is not affected by this hormone. We decided,

TABLE I. LPL Activity in Hearts of Sham Operated, Thyroidectomized, Adrenalectomized and Adrenal-Demedullated Rats Maintained at Room Temperature and Exposed to 4°.[a]

		Heart weight (mg)	LPL units/g wet wt	LPL units/ heart
Sham operated*	Room temperature	437 ± 19	160 ± 24	70 ± 11
	4°	414 ± 36	242 ± 24**	100 ± 12**
Thyroidectomized	Room temperature	351 ± 34	236 ± 30	82 ± 4
	4°	359 ± 25	312 ± 8**	109 ± 6**
Adrenalectomized	Room temperature	427 ± 19	65 ± 17	28 ± 9
	4°	399 ± 19	137 ± 19**	55 ± 9***
Adrenal-demedullated	Room temperature	410 ± 57	87 ± 15	36 ± 9
	4°	443 ± 25	156 ± 24**	69 ± 12**

[a] Groups of 5 rats were starved overnight and exposed to 4° for 3 hr.

* 5 sham thyroidectomized plus 5 sham adrenalectomized.

** $P < 0.001$.

*** $P < 0.005$ when compared to animals maintained at room temperature.

TABLE II. Effect of Insulin on LPL Activity of Heart and Adipose Tissue of Rats Maintained at Room Temperature and Exposed to 4°.[a]

		LPL units/g wet wt	
	Insulin	Heart	Adipose tissue
Room temperature	—	126 ± 30	44 ± 12
Room temperature	+	159 ± 12	209 ± 44*
4°	—	205 ± 29**	61 ± 24
4°	+	216 ± 29	197 ± 35*

[a] Groups of 5 rats which had been starved overnight were injected intraperitoneally with either saline or 0.4 units of insulin per kilogram of body wt. Immediately after the injections some animals were exposed to 4°. All animals were sacrificed 3 hr later.

* $P < 0.001$, when compared to controls.

** $P < 0.01$, when compared to animals maintained at room temperature.

therefore, to compare the effects of insulin administration on the LPL activity of hearts from starved rats exposed to 4° or maintained at room temperature. As shown in Table II administration of 0.4 units of insulin/kg body wt had no effect on the myocardial enzyme activity in both groups of rats. This dose was sufficient, however, to induce a significant change in the LPL activity of the adipose tissue of both groups of animals.

Discussion. The results of the present study confirm previous reports (7, 8, 12) that exposure of normal rats to cold temperatures for short periods induces a significant rise in myocardial LPL activity. The finding that the diaphragms of the cold exposed animals also showed significant increments in LPL activity is in agreement with previous conclusions (5, 9) that the LPL activities of heart and skeletal muscle may have similar regulatory mechanisms. In this study, we measured mainly changes in heart LPL activity because, in this tissue, high enzyme activities can be easily determined in aqueous homogenates while enzyme activities in skeletal muscle can be detected only in time consuming preparations of defatted tissue (5). Furthermore, in the heart, LPL activity per unit wt is considerably higher than that of skeletal muscle and, therefore, changes in activity can be more readily detected.

The increase in LPL activity in the heart and skeletal muscle of cold exposed rats is an important metabolic adaptation which allows these tissues to derive a considerable portion of their increased energy requirements from the oxidation of plasma TGFA. Radomski (19) reported that the exposure of rats to 4° is accompanied by a rapid reduction in the concentration of the TG rich very low density lipoprotein fraction of the plasma. McBurney and Radomski (20) subsequently concluded that this reduction in circulating very low density lipoproteins was due, at least in part, to the increased uptake by the tissues, of TGFA from this lipoprotein fraction. Rogers *et al.* (8), using the isolated perfused rat heart preparation, obtained direct evidence that hearts from rats exposed to 4° for 3 hr utilize significantly more lipoprotein TGFA than the hearts of animals maintained at room temperature. Moreover, this increased utilization of TGFA was directly related to the increased LPL activity in the hearts of the animals exposed to 4°.

The factors which cause the increase in LPL activity in the muscles of rats exposed to cold temperatures remain to be identified. The results of the present study clearly indicate that adrenal and thyroid hormones are not necessary for this process. The results obtained with the adrenal-demedullated animals, on the other hand, suggest that catecholamines may be important for the maintenance of normal levels of enzyme activity in the heart. It seems reasonable to argue, in the light of these results, that the

increases in heart LPL activity, following the injection of catecholamines and thyroid hormone reported in other studies (7, 10, 11), may have been due to indirect effects mediated by changes in plasma or tissue metabolites which could then affect the activity of the enzyme.

The reasons for the discrepancy between our results (Table II and Reference 5) and those of Radomski and Orme (7) who found that insulin administration decreases heart LPL activity, require further investigation. One possible explanation is that the experimental conditions used in the present study and in that of Radomski and Orme were not comparable. These authors used rats which were maintained in the cold for 48 hr and, during this period, injected with a total of 15 units of insulin/kg body wt while in the present study the animals were exposed to 4° for only 3 hr and injected with 0.4 units of insulin/kg body wt. Another possible explanation is that in the studies of Radomski and Orme the nutritional status of the control animals was different from that of the animals injected with insulin. Thus, these authors injected insulin into rats which had free access to food. Because injections of insulin are accompanied by hyperphagia (21, 22), it is possible that the control animals were "fasted," i.e., with a higher myocardial LPL activity (5, 6), compared to the insulin injected animals.

Summary. Starved thyroidectomized, adrenalectomized, adrenal-demedullated and sham operated rats were exposed to 4° for 3 hr and their heart lipoprotein lipase activities compared to those of animals maintained at room temperature. The lipoprotein lipase activity in the hearts of the thyroidectomized rats was comparable to that of the sham operated animals when expressed as units/heart. The enzyme activities in the hearts of the adrenalectomized and adrenal-demedullated rats were about 50% lower than those of the controls. In all animals, exposure to cold temperature induced significant increases in the myocardial lipoprotein lipase activity. In normal rats this effect could not be reversed by the administration of insulin. It is concluded that the increases in lipoprotein lipase activity in the hearts of rats exposed to cold temperatures are not dependent on adrenal and thyroid hormones.

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