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The Inhibition *in vivo* of Lipoprotein Lipase (Clearing-Factor Lipase) Activity by Triton WR-1339

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1. Lipoprotein lipase activity was measured in heart homogenates and in heparin-releasable and non-releasable fractions of isolated perfused rat hearts, after the intravenous injection of Triton WR-1339. 2. In homogenates of hearts from starved, rats, lipoprotein lipase activity was significantly inhibited ($P < 0.001$) 2h after the injection of Triton. This inhibition was restricted exclusively to the heparin-releasable fraction. Maximum inhibition occurred 30min after the injection and corresponded to about 60% of the lipoprotein lipase activity that could be released from the heart during 30s perfusion with heparin. 3. Hearts of Triton-treated starved rats were unable to take up and utilize ^{14}C-labelled chylomicron triacylglycerol fatty acids, even though about 40% of heparin-releasable activity remained in the hearts. 4. It is concluded that Triton selectively inhibits the functional lipoprotein lipase, i.e. the enzyme directly involved in the hydrolysis of circulating plasma triacylglycerols. 5. Lipoprotein lipase activities measured in homogenates of soleus muscle of starved rats and adipose tissue of fed rats were decreased by 25 and 39% respectively after Triton injection. It is concluded that, by analogy with the heart, these Triton-inhibitable activities correspond to the functional lipoprotein lipase.

When the non-ionic detergent Triton WR-1339 is injected intravenously into experimental animals, there is a progressive accumulation of triacylglycerols in the plasma (Friedman & Byers, 1953; Otway & Robinson, 1967). This effect has been interpreted as being due to the inability of lipoprotein lipase present in the extrahepatic tissues to hydrolyse the plasma triacylglycerols of Triton-treated animals (Scanu & Oriente, 1961; Scanu *et al.*, 1961; Otway & Robinson, 1967). Experiments with plasma from rats treated with heparin led Schotz *et al.* (1957) to conclude that this inhibition of triacylglycerol hydrolysis is due to the coating of the plasma lipoproteins by the detergent. This would prevent the interaction of substrate and lipoprotein lipase which normally takes place at or close to the lumenal surface of the capillaries (Borensztajn & Robinson, 1970; Robinson, 1970). In the course of studies on the metabolism of rat plasma triacylglycerols *in vivo* we observed that Triton also has a direct inhibitory effect on the lipoprotein lipase activity of muscle and of adipose tissue. We have examined this phenomenon further and report evidence that Triton inhibits selectively that lipoprotein lipase that is directly involved in the hydrolysis of circulating triacylglycerols *in vivo*.

Materials and Methods

Animals

Male Sprague–Dawley rats (180–220g) were maintained on a laboratory chow diet [Teklad 4% (w/w) fat; ARS–Sprague–Dawley, Winfield, IA, U.S.A.]. They were starved for 15h before each experiment. In some cases, the starved rats, while under diethyl ether anaesthesia, were force-fed with 3ml of water or a 60% (w/v) solution of glucose, 3h before the experiments. All animals received intravenous injections of 1ml of 0.9% NaCl or 1ml of a 10% (v/v) solution in 0.9% NaCl of Triton WR-1339 (oxyethylated t-octylphenol polymethylene polymer; Ruger Chemical Co., Irvington, NJ, U.S.A.). They were killed by removing their hearts after partial exsanguination through the abdominal aorta, while under diethyl ether anaesthesia.

Measurements of lipoprotein lipase activity

To measure heparin-releasable lipoprotein lipase activity, hearts were dissected out and perfused for 30s in a non-recirculatory system with Krebs–Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932). They were then perfused for 30s with the same buffer containing 1% (v/v) rat serum and 5 units of heparin/ml (Borensztajn & Robinson, 1970). Preliminary experiments showed that during the 30s perfusion period with buffer only, no detectable lipoprotein lipase activity was released from the heart. The enzyme activity was therefore measured only in the heparin-containing perfusate. To measure the non-releasable lipoprotein lipase activity, the hearts were perfused with heparin and were then homogenized in 0.025M-NH_3/NH_4Cl buffer (pH 8.1) by using a Duall ground-glass tissue grinder (Kontes Glass Co., Chicago, IL, U.S.A.), and 2ml portions of this homogenate were

added to the assay medium. To measure lipoprotein lipase activities of non-perfused hearts, soleus muscle and epididymal fat-pads, these tissues were also homogenized by the above procedure. The final concentration of all homogenates was 50mg of tissue/ml. The composition of the lipoprotein lipase assay medium and the procedures for the incubation, extraction and measurement of unesterified fatty acids released into the assay medium have been described (Borensztajn *et al.*, 1972). The lipolytic activity in the perfusates and in the homogenates had the characteristics of lipoprotein lipase, e.g. the reaction was inhibited by more than 93% in the presence of 1M-NaCl. The enzyme 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.

Perfusions with ^{14}C-labelled chylomicrons

To measure the capacity of control and of Triton-treated rat hearts to take up and utilize ^{14}C-labelled triacylglycerol fatty acids, hearts were perfused for 1min with Krebs–Ringer bicarbonate buffer (pH7.4) and then for 20min with the same buffer containing 0.45 μequiv. of ^{14}C-labelled chylomicron triacylglycerol fatty acids/ml. The methods for the collection and washing of the labelled chylomicrons to decrease the amount of ^{14}C-labelled unesterified fatty acids have been described previously (Borensztajn & Robinson, 1970). The techniques of perfusion and the methods to measure the $^{14}CO_2$ produced by the perfused hearts and the ^{14}C label incorporated into the heart lipids at the end of the perfusion have been described (Enser *et al.*, 1967).

Chemicals

Sodium heparin was purchased from the Upjohn Company, Kalamazoo, MI, U.S.A. [1-^{14}C]Palmitic acid (57.9 mCi/mmol), was purchased from Amersham–Searle Corp., Chicago, IL, U.S.A.

Results

Heart lipoprotein lipase activity of Triton-injected rats

The results in Table 1 show that, under the assay conditions used, the lipoprotein lipase activities in the hearts of starved rats were decreased by about 28% ($P < 0.001$) 2h after the Triton injection. Since small amounts of Triton, trapped in the tissue vascular bed, were inevitably carried with the homogenates to the assay medium, the decrease in lipoprotein lipase activity might have been due to the association of the detergent with the substrate, as suggested by Schotz *et al.* (1957). This possibility was ruled out, however, since under identical experimental conditions the injection of Triton had no detectable effect on the heart lipoprotein lipase activity of fed rats (Table 1).

Heparin-releasable and non-releasable lipoprotein lipase activities of hearts from Triton-injected rats

In the heart and in other extrahepatic tissues, two fractions of lipoprotein lipase activity can be distinguished according to their property of being readily released from the tissue by heparin, namely a heparin-releasable and a non-releasable fraction (Borensztajn & Robinson, 1970; Robinson, 1970). The increase in lipoprotein lipase activity observed in the hearts of starved rats, compared with fed, occurs primarily in the heparin-releasable fraction (Borensztajn & Robinson, 1970; Borensztajn *et al.*, 1975). The finding that Triton injected *in vivo* inhibited the lipoprotein lipase activity in the hearts of starved rats only (Table 1) suggested that Triton acts primarily on the heparin-releasable lipoprotein lipase fraction. Triton was injected into starved rats and, at different time-intervals, the heart heparin-releasable and non-releasable lipoprotein lipase activities were measured. As shown in Fig. 1, at 5min after the injection no significant ($P > 0.05$) differences in activities were observed in either fraction, compared with the control animals. After 15min, the activity of the heparin-releasable fraction had decreased to about 70% ($P < 0.001$) of the control and by 30min only about 40% ($P < 0.001$) of the original activity remained. No further decreases in activity were observed after 30min. In other experiments (J. Borensztajn, M. S. Rone & T. J. Kotlar, unpublished work) it was shown that this progressive decrease in activity was not due to the release of the enzyme from the tissue by the detergent. In contrast with the heparin-releasable fraction, the non-releasable lipoprotein lipase activity was not affected by Triton administration (Fig. 1).

Table 1. *Effect of Triton injection on the lipoprotein lipase activity of fed and starved rat hearts*

Rats were starved for 15h and were then force-fed with 3ml of water (starved) or 3ml of a 60% (w/v) solution of glucose (fed) 3h before the intravenous injection of 1ml of 0.9% NaCl or 1ml of a 10% (v/v) solution of Triton. After 2h the animals were killed and the lipoprotein lipase assays carried out as described in the Materials and Methods section. Results are means ± S.E.M. for the numbers of animals shown in parentheses.

	Heart lipoprotein lipase activity (units)	
Injection	Fed	Starved
NaCl	78 ± 3 (5)	166 ± 3 (9)
Triton	77 ± 2 (5)	119 ± 4 (9)

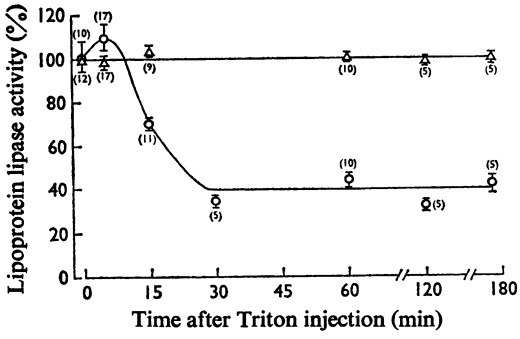

Fig. 1. *Heparin-releasable and non-releasable lipoprotein lipase activities from hearts of control and Triton-injected rats*

Rats that had been starved for 15h were injected with 1ml of 10% (v/v) Triton solution in 0.9% NaCl. At the times indicated, their hearts were isolated and were perfused for 30s with Krebs–Ringer bicarbonate buffer (pH7.4) and then for 30s with this same buffer containing 1% (v/v) rat serum and 5 units of heparin/ml. The perfusates containing serum and heparin were collected for the assay of the heparin-releasable lipoprotein lipase activity (○). The non-releasable enzyme activity (△) was measured in the hearts at the end of the perfusion. The lipoprotein lipase assays were carried out as described in the Materials and Methods section. The activities of both enzyme fractions of control hearts are expressed as 100%. The numbers of hearts used at each time-point are shown in parentheses.

In fed rat hearts, the heparin-releasable lipoprotein lipase activity is very low (Borensztajn & Robinson, 1970). It was therefore difficult to detect, in these hearts, significant decreases in the activity of this fraction after Triton injection.

Utilization of [1-^{14}C]palmitate-labelled chylomicron triacylglycerol fatty acids by hearts of Triton-injected rats

Studies with the isolated perfused rat heart (Borensztajn & Robinson, 1970) have shown that removal of the heparin-releasable lipoprotein lipase fraction results in the inability of the tissue to hydrolyse plasma lipoprotein triacylglycerols. Since in the hearts of rats injected with Triton for 30min or longer 40% of the heparin-releasable activity was still present (Fig. 1), we investigated the extent to which these hearts could utilize chylomicron triacylglycerols. As shown in Table 2, hearts from rats injected with Triton 5min before being killed, in which the lipoprotein lipase activity was not decreased (Fig. 1), were able to oxidize and incorporate into the tissue lipids the same amounts of ^{14}C-labelled triacylglycerol fatty acids as the control hearts. The hearts of rats injected with Triton 60min before being killed, on the other hand, oxidized and incorporated into the tissue

Table 2. *Utilization of [1-^{14}C]palmitate-labelled chylomicron triacylglycerol fatty acids by hearts of Triton-injected rats*

Hearts from NaCl- or Triton-injected starved rats were perfused for 60s with Krebs–Ringer bicarbonate buffer (pH7.4) and then for 20min with the same buffer containing washed ^{14}C-labelled chylomicrons (0.45μequiv. of triacylglycerol fatty acids/ml). The chylomicron-containing perfusates were collected for $^{14}CO_2$ measurements. In one group, hearts were perfused for 30s with Krebs–Ringer bicarbonate buffer containing 1% (v/v) rat serum and 5 units of heparin/ml after the 60s wash-out period and before the 20min perfusion with chylomicrons. At the end of each perfusion, the hearts were further perfused with 20ml of ice-cold buffer alone, and weighed pieces of the ventricles were taken to measure the ^{14}C label incorporated into the tissue lipids. Results are means ± S.E.M. for the numbers of hearts perfused in each group shown in parentheses.

Time after Triton injection (min)		Triacylglycerol fatty acids utilized (μequiv./g wet wt.)		
		Oxidized to $^{14}CO_2$	^{14}C label incorporated into heart lipids	Total ^{14}C label utilized
0 (control)	(9)	1.39 ± 0.07	0.83 ± 0.09	2.22 ± 0.13
5	(5)	1.39 ± 0.04	0.83 ± 0.09	2.22 ± 0.009
60	(4)	0.37 ± 0.1	0.19 ± 0.005	0.56 ± 0.1
60 (perfused with heparin)	(4)	0.32 ± 0.03	0.18 ± 0.01	0.50 ± 0.03

lipids only about 25% ($P < 0.001$) as much of the ^{14}C label as the control hearts. This amount of ^{14}C label utilized by the hearts of the Triton-treated rats can be accounted for by the uptake of the small quantities of ^{14}C-labelled unesterified fatty acids present in the perfusion medium rather than by hydrolysis and uptake of ^{14}C-labelled chylomicron triacylglycerols (Borensztajn & Robinson, 1970). This interpretation is consistent with the finding that the removal by heparin of the residual 40% of heparin-releasable lipoprotein lipase activity from the hearts of the Triton-treated animals did not result in further decreases in $^{14}CO_2$ formation or incorporation of ^{14}C label into the tissue lipids (Table 2). These results indicate that only that portion of the total heart lipoprotein lipase activity that was inhibited by Triton is directly involved in the hydrolysis of plasma triacylglycerols.

Lipoprotein lipase activity in adipose tissue and soleus muscle of Triton-injected rats

The results of Table 3 show that 60min after the injection of Triton into fed rats the adipose-tissue

Table 3. *Lipoprotein lipase activity in adipose tissue and in soleus muscle of fed and starved Triton-injected rats*

Rats were starved for 15h and then force-fed with 3ml of water or 3ml of a 60% (w/v) glucose solution. After 3h they were injected with 1ml of 0.9% NaCl or 1ml of a 10% (v/v) Triton solution and killed 2h later. The lipoprotein lipase assays were carried out as described in the Materials and Methods section. Results are means ± S.E.M. for the numbers of animals shown in parentheses.

	Lipoprotein lipase activity (units)	
Injection	Adipose tissue (fed)	Soleus muscle (starved)
NaCl	139 ± 10 (9)	44 ± 2 (9)
Triton	85 ± 10 (9)	33 ± 2 (9)

lipoprotein lipase activity was decreased by about 39% compared with that of controls ($P < 0.005$). In starved rats the injection of Triton caused a 25% decrease in the soleus-muscle lipoprotein lipase activity ($P < 0.005$).

Discussion

On the basis of experiments using the isolated rat heart, it had been previously concluded (Borensztajn & Robinson, 1970) that the heart functional lipoprotein lipase, i.e. the enzyme in the intact tissue directly involved in the hydrolysis of plasma triacylglycerols, is represented by that portion of the total lipoprotein lipase activity that can be released by perfusion with heparin in an arbitrarily set period of time. The results of the present study show, however, that only a portion of the heparin-releasable fraction can be considered truly functional. Thus 60min after the administration of Triton to starved rats, when about 40% of the lipoprotein lipase activity that could be released by heparin within a 30s period was still present (Fig. 1), the capacity of the hearts to hydrolyse chylomicron triacylglycerols was virtually abolished (Table 2). The hydrolysis of plasma chylomicron and very-low-density lipoprotein triacylglycerols occurs at or close to the lumenal surface of the endothelial cells of the capillaries (Schoefl & French, 1968; Borensztajn & Robinson, 1970; Blanchette-Mackie & Scow, 1971). It can therefore be concluded, on the basis of the present results, that the inhibitory effect of Triton on heart lipoprotein lipase is most probably due to its interaction with the enzyme present on the endothelial surface. It remains to be explained how heparin can release more than the functional (Triton-inhibitable) lipoprotein lipase activity. It is possible that Triton, a polymer that rapidly associates with relatively large plasma lipoproteins (Scanu & Oriente, 1961; Otway & Robinson, 1967), has its access limited only to the lipoprotein lipase of the endothelial surface. Heparin, on the other hand, being a much smaller molecule, has access not only to the enzyme that can be inhibited by Triton, but also to the lipoprotein lipase that is present in sites not accessible to the detergent. That lipoprotein lipase is present in different sites of the heart tissue is supported by studies (Chajek *et al.*, 1975; Borensztajn *et al.*, 1975) that have shown that this enzyme is transported from its site of synthesis, presumably the myocardial cell, to the surface of the endothelium. It is therefore possible that heparin releases from the tissue the functional enzyme and, in addition, some of the lipoprotein lipase which is in the process of being transported to the endothelial surface.

Another possible explanation why Triton inhibits only part of the total heparin-releasable lipoprotein lipase fraction is that heparin may activate and then release a non-active form of the enzyme present on the endothelial surface which is not inhibited by Triton. The mechanisms whereby heparin acts by releasing and/or activating lipoprotein lipase from the tissue and also how Triton inhibits the enzyme remain to be investigated.

The experiments using isolated hearts (Fig. 1 and Table 2) showed that only a fraction of the total tissue lipoprotein lipase activity is functional. In non-perfused organs, the functional enzyme activity can also be readily measured. The difference in total lipoprotein lipase activity in homogenates of hearts from rats injected with 0.9% NaCl or Triton represents this functional enzyme (Table 1). Since it is assumed that, as in the heart, the uptake of plasma triacylglycerol fatty acids by most extrahepatic tissues depends on the lipoprotein lipase activity present on the endothelial surface (Robinson, 1970), the rationale used above for the heart, i.e. equating functional lipoprotein lipase activity with Triton-inhibited lipoprotein lipase activity, can be extended, by analogy, to other extrahepatic tissues. Thus in the soleus muscle of starved rats, under the assay conditions used, the functional enzyme represents about 25% of the total activity, whereas in the adipose tissue of fed rats it represents about 39% of the total activity (Table 3). It is noteworthy that Cunningham & Robinson (1969), using an enzyme assay system similar to that of the present study, found that in the adipose tissue of fed rats about 80% of the intact tissue lipoprotein lipase is localized outside the adipocytes. In the light of the present results it would seem that about 40% of the intact tissue lipoprotein lipase is localized between the adipocytes and the endothelial surface, presumably in the process of being transported from its site of synthesis to its site of action.

The amount of Triton administered to the rats in the present study was sufficient to cause a marked accumulation of triacylglycerols in the plasma. By 1h after the Triton injection the concentration of plasma triacylglycerols had increased from 36 ± 3

to 202 ± 18 mg/100ml of plasma (mean $\pm$ S.E.M., $n = 5$). Although the coating of the plasma lipoproteins by the detergent is very likely an important factor responsible for this phenomenon (Schotz *et al.*, 1957; Scanu & Oriente, 1961; Scanu *et al.*, 1961), the results of the present investigation clearly indicate that the rapid inhibition of the functional lipoprotein lipase activity of muscle and adipose tissue could also contribute to the development of the hypertriglyceridaemia.

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