The Effect of Adrenergic Agents on the Activities of Glycerol 3-phosphate Acrlytransferase and Triglyceride Lipase in the Isolated Perfused Rat Heart

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G. P. HEATHERS, N. AL-MUHTASEB AND R. V. BRUNT. The Effect of Adrenergic Agents on the Activities of Glycerol 3-phosphate Acrlytransferase and Triglyceride Lipase in the Isolated Rat Heart. Journal of Molecular and Cellular Cardiology (1985) 17, 785-796. Glycerol 3-phosphate acryltransferase (GPAT) activity and triglyceride lipase (TGL) activity were measured in homogenates from hearts perfused with adrenergic agonists and antagonists. Perfusion with adrenalin or the β-agonist isoprenaline produced an increase in TGL activity and a fall in GPAT activity. These changes could be mimicked by incubation of heart homogenates with cAMP-dependent protein kinase. The α₁-agonist clonidine produced the opposite effect, thus it increased GPAT activity and decreased TGL activity. Methoxamine, an α₂-agonist, had no effect on TGL activity but reduced GPAT activity. Continuous perfusion of the β-agonist atenolol reduced TGL activity to half that found in controls but also reduced GPAT activity. No change was seen on continuous perfusion of α₁- or α₂-agonists. Changes in GPAT activity were localized mainly in the microsomal enzyme. These changes are consistent with both enzymes being regulated via a cyclic-AMP dependent protein kinase system and via α-adrenergic mechanisms.

Key Words: Adrenalin; Clonidine; Methoxamine; Isoprenaline; Atenolol; Glycerol 3-phosphate acryltransferase; Triglyceride lipase; cAMP-dependent protein kinase; Triglyceride mobilization.

Introduction

Triglycerides, both endogenous and exogenous, are utilized to support energy metabolism in the heart. Under most conditions, fatty acids are oxidized in preference to carbohydrate and their oxidation normally accounts for 60% to 70% of oxidative metabolism [47]. In rat hearts perfused with glucose as the only exogenous energy source, the oxidation of glucose could only account for approximately 40% of oxygen consumption suggesting that endogenous lipids were also utilized in preference to carbohydrate [47].

A number of investigators have provided evidence that catecholamines cause a concurrent increase in pressure development in the working rat heart and an increase in the rate of endogenous triglyceride mobilization, as demonstrated by increased rates of glycerol output [17, 37] and decreased tissue levels of total [22, 26] and isotopically pre-labelled [22] triglycerides. Major determinants of the rate of triglyceride mobilization should be the competing activities of the lipolytic and esterification pathways and in particular the activities of their initial rate limiting enzymes, neutral triglyceride lipase (glycerolester hydrolase, EC3.1.1.3) and glycerol 3-phosphate acyl transferase (acyl-coA-sn-glycerol 3-phosphate 1-acyltransferase, EC2.3.1.15).

Catecholamines have been shown to increase the rate of lipolysis in rat [37], chick [35] and human [15, 39] adipose tissue by increasing the activity of triglyceride lipase (TGL). Stimulation of TGL activity occurs by catecholamine activation of adenyl cyclase, the consequent increase in cAMP levels, increased cAMP-dependent protein kinase activity and the phosphorylation of TGL [20, 30]. Catecholamines can interact with four different receptors on the cell surface; α₁, α₂,
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\( \beta \) and \( \beta_2 \)-adrenergic receptors [9, 62]. In adipose tissue, activation of the \( \beta \)-receptors mediates the increase in cAMP levels, via activating membrane-bound adenylyl cyclase, resulting in increased lipolysis after phosphorylation of triglyceride lipase [4, 25, 32]. Activation of \( \beta_2 \) receptors decreases cAMP concentration and reduces cAMP-dependent protein kinase activity [32]. Indeed activation of \( \beta_2 \) receptors has been shown to reduce cAMP levels and the rate of lipolysis in human and hamster (but not rat) adipose tissue [13, 14]. No effect of activation or antagonism of \( \beta_1 \) receptors on the rate of lipolysis has been shown [32].

In the heart no activation of a neutral triacylglyceride lipase by catecholamines has yet been reported [97]. Although Palmer and coworkers [44] have shown adrenaline activation of a cardiac lipase with the characteristics of lipoprotein lipase.

The esterification pathway is also under hormonal control [24, 30]. Sooranna and Saggerson have shown a fall in GPAT activity in rat adipocytes after exposure to adrenaline or noradrenaline [31, 32]. This may be blocked by the \( \beta \)-adrenergic antagonist propranolol. Perfusion of rat liver with dibutyryl adenosine-3',5'-monophosphate reduces esterification at a step prior to the formation of phosphatidate [49]. There have been contradictory reports on the effect of cAMP-dependent protein kinase in GPAT activity. Nimmo and Houston found a time-dependent inactivation of the activity of rat adipose GPAT [43] while no such inactivation could be found by Rider and Saggerson [45].

Glycerol 3-phosphate acyltransferase (GPAT) is found in both the microsomes and the mitochondria of most mammalian tissues including the rat heart [27]. A number of differences between the two forms have been found [38, 42] not least the difference in sensitivity to sulphonylurea reagents [27]. The ability of N-ethyl maleimide to inhibit the microsomal enzyme but not the mitochondrial form has been used in the past to distinguish between the two forms [2, 3]. Although some hormonal effects have been seen with mitochondrial GPAT [45], most attention has been given to the microsomal form [5] as there is general agreement that the principal site of triglyceride synthesis is the endoplasmic reticulum.

This paper provides evidence for adrenergic control of intracellular triglyceride mobilization in the heart. Changes in the activity of TGL and GPAT under catecholamine stimulation are shown and a likely role for cAMP and cyclic AMP-dependent protein kinase is discussed.

Methods and Materials

Heart perfusion

Hearts were perfused at 37°C by a non-recirculating Langendorff system. Coronary flow was generated with a perfusion pump. The perfusion was a modified Krebs Henseleit bicarbonate buffer (pH 7.4) containing NaCl (118.4 mm), KCl (4.6 mm), MgSO₄ (1.1 mm), NaH₂PO₄ (1.0 mm), CaCl₂ (2.5 mm), glucose (11.1 mm) and NaHCO₃ (24.9 mm). This was gassed with 95% O₂/5% CO₂.

Male Wistar rats (approximately 200 g) were fed ad libitum on laboratory chow (oxoid 41B). They were anaesthetized by intraperitoneal injection of a mixture of sodium pentobarbitone ("Sagatal", May and Baker, Dagenham, England) (80 mg/kg) and heparin (Evans Medical, Liverpool, England) (Na salt, 2400 units/kg). The heart was quickly excised and placed in ice-cold modified Krebs Henseleit buffer, while extraneous (mainly lipid) tissue was removed and a cannula inserted into the aorta. The heart was then mounted on the perfusion apparatus and attached to a U22 dynamometer strain gauge, resting and developed tension being recorded continuously on a Devices MX2 recorder. Heart rate was measured manually using a stopwatch.

After 20 min a solution of the adrenergic agonist was introduced into the system to give a final concentration of 10⁻⁶ M. The peak effect of any change in developed tension and/or heart rate was evident between 1 and 2 min after the agonist was introduced. At this time the hearts were quickly removed and homogenized (at mk3 for 5 s) by a Polytron homogenizer (Northern Media Supply Ltd) in ice-cold buffer containing 0.25 M sucrose, 10 mM Tris, 1 mM EDTA at pH 7.6.

Adrenergic antagonists were added to the perfusate after 10 minutes perfusion and remained present for a further 10 min before the heart was removed and homogenized.
The homogenate was then either used directly as a crude glycerolphosphate acyl transferase preparation or centrifuged at 10,000 g for 10 min at 4°C in a microfuge. The supernatant was used as a crude triglyceride lipase preparation.

**Enzyme assay**

Glycerol 3-Phosphate acyl transferase (GPAT) was assayed at 30°C in a final volume of 100 μl containing 0.25 m sucrose, 10 mM Tris, 50 mM KCl, 1 mM EDTA, at pH 7.4 with 5 mM (U-14C)Glycerol 3-Phosphate (0.1 μCi), 100 μM Palmitoyl CoA and 0.2 mg of fatty acid poor bovine serum albumin [16]. The reaction was initiated by addition of 10 μl of homogenate (approximately 50 μg of protein). After 5 min the reaction was stopped by addition of 300 μl of TCA saturated butanol. After vortexing, the layers were separated by centrifugation in an Eppendorf microfuge for 2 min. The aqueous layer was removed while the butanol layer was washed with 750 μl of 10% trichloroacetic acid (TCA). A 100 μl sample of the butanol layer was dissolved in scintillation fluid (600 ml of Triton, 10 g of PPO, 1400 ml of toluene). Each time point was done in triplicate. The radioactivity was counted in a Tricarb liquid scintillation counter (Packard Instruments Ltd). Preliminary experiments had shown that this procedure extracted only acylglycerophosphatides of which 97% were diacyl glycerophosphate. Results are expressed as nmoles of glycerol 3-phosphate esterified/min/mg of protein.

Triglyceride lipase (TGL) was assayed by the method of Severson [37] with some modification. 3 μl (15 nCi) of an ethanolic dispersion of (U-14C)Triolein was added to 67 μl of 50 mM sodium phosphate buffer pH 7.4 (with 0.2% w/v fatty acid poor albumin) to give a final triolien concentration of 4 mM. This suspension was then sonicated for 30 s in a sonication bath (Dawg Instruments Ltd) immediately before use. The reaction was initiated by addition of 10 μl of the 10,000 g supernatant (approximately 30 μg of protein). After 30 min incubation at 30°C, the reaction was terminated by addition of 300 μl of a fatty acid extraction solution [4] containing methanol : chloroform : heptane (1.41:1.25:1) and 0.1 mM of carrier Oleic acid followed by 10 μl of 1 N NaOH. After vortexing vigorously, the layers were separated by centrifugation in a microfuge. 50 μl of the upper aqueous layer was transferred to a vial containing 4 ml of scintillation fluid and 5 μl of 1 N HCl. Each time point was done in triplicate and radioactivity was measured as before. Results are expressed as nmoles of fatty acid released/min/mg of protein.

**Incubation with cAMP-dependent protein kinase**

Cyclic-AMP-dependent protein kinase was prepared from rabbit skeletal muscle by the method of Walsh et al. [67] up to and including the DE 52 ion exchange chromatography. Freshly perfused heart homogenate or supernatant (approximately 0.5 mg) was incubated at 30°C for 15 min in buffer (0.25 m sucrose, 10 mM Tris, 10 mM MgCl₂, 1 mM EDTA pH 7.6) with, when included, 5 mM ATP, 5 μM cAMP, and 0.1 mg of the rabbit skeletal muscle cAMP-dependent protein kinase in a final volume of 200 μl. Samples were then assayed immediately after incubation for their activity of GPAT (homogenate) or TGL (supernatant).

**Incubation under dephosphorylation conditions**

The method used here is based on the method of Severson et al. [42]. It depends on the activation by Ca²⁺ and Mg²⁺ of endogenous phosphoprotein phosphatase. A final volume of 1 ml contained 500 μl of homogenate or supernatant (approximately 2.5 mg of protein), MgCl₂ (10 mM), CaCl₂ (12 mM), EGTA (10 mM), MOPS (3-(N-Morpholino)propanesulphonic acid) (20 mM, pH 7.4) and one drop of mercaptoethanol. The preparations were incubated at 30°C for 30 min, after which they were assayed for GPAT activity (homogenate) or TGL activity (supernatant). Control preparations contained every reagent except MgCl₂ and CaCl₂.

Protein was measured by the Coomassie Blue method of Bradford [12] using bovine serum albumin as standard.

**Materials**

Laboratory chemicals, unless otherwise stated, were Analar and were from BDH Ltd, Poole, England.
Sigma Chemical Co. (Kingston upon Thames, Surrey, England) supplied glycerol 3-phosphate, palmitoyl coA, bovine serum albumin, adrenalin, clonidine, isoprenaline, atenolol, tolbutamide. Radio-labelled chemicals were from The Radiochemical Centre, Amersham, England. Methoxamine was a gift from Welcome Research, Beckenham, England. Doxazosin was a gift from Pfizer Central Research, Sandwich, England.

Results

The activities of the enzymes triglyceride lipase (TGL) and glycerol 3-phosphate acyltransferase (GPAT) against time are shown in

![Figure 1](image1.png) **Figure 1.** The effect in incubation time on the TGL activity. No. of measurements = 6; values are means ± S.E.M.

![Figure 2](image2.png) **Figure 2.** The effect of the enzyme concentration on FA released. No. of observations = 6; values are means ± S.E.M.

![Figure 3](image3.png) **Figure 3.** Effect of incubation time on GPAT activity. No. of observations = 6; values are means ± S.E.M.

![Figure 4](image4.png) **Figure 4.** Effect of GPAT concentration on acylation. No. of observations = 6; values are means ± S.E.M.

Figures 1 and 3, Figures 2 and 4 show the activities of TGL and GPAT against the amount of protein added to the particular assay. In subsequent assays for the activity of GPAT a time of 5 min and the addition of 50 ± 10 µg of homogenate protein were standard. The standard assay for the measurement of TGL activity involved a time course of 30 min with an addition of 30 ± 5 µg of supernatant protein.

**Effect of adrenalin infusion**

When adrenalin was added at any of the three concentration shown, the expected increase in inotropy and chronotropy was seen.
Adrenergic Control of GPAT and TGL Activities

### TABLE 1. The effect of adrenaline on the activity of glycerol 3-phosphate acyl transferase and triglyceride lipase

<table>
<thead>
<tr>
<th>Enzyme activity (nmol/min/mg protein)</th>
<th>GPAT</th>
<th>TGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>[4] 0.63 ± 0.07</td>
<td>[5] 0.20 ± 0.03</td>
</tr>
<tr>
<td>Adrenaline (10^{-8} M)</td>
<td>[3] 0.52 ± 0.03^a</td>
<td>[3] 0.27 ± 0.02^a</td>
</tr>
<tr>
<td>Adrenaline (10^{-6} M)</td>
<td>[6</td>
<td>0.30 ± 0.05^b</td>
</tr>
<tr>
<td>Adrenaline (10^{-4} M)</td>
<td>[7] 0.34 ± 0.02^c</td>
<td>[5] 0.32 ± 0.04^b</td>
</tr>
<tr>
<td>10 min after peak adrenaline phase</td>
<td>[6] 0.60 ± 0.03</td>
<td>[6] 0.18 ± 0.02</td>
</tr>
</tbody>
</table>

Enzyme activities are expressed as nmols of glycerol 3-phosphate esterified/min/mg of homogenate protein (GPAT) and nmols of fatty acid released/min/mg of supernatant protein (TGL).

Values are expressed as (no. of observations) ± s.e.m.

Statistical significance compared with control values are: control v. 1, n.s.; control v. 2, P < 0.001; control v. 3, P < 0.01; control v. 4, P = 0.05; control v. 5, P < 0.05; control v. 6, P = 0.01.

Maximum effect was noted 1½ min after the addition. Hearts were removed at the same time as the peak effect or 10 min after, rapidly homogenized and the activities of both GPAT and TGL measured. It was found that at the time of peak mechanical response TGL activity had increased to 150% and GPAT activity decreased to 50% of control values when perfused with 10^{-8} M or 10^{-4} M adrenaline (Table 1). At 10^{-8} M adrenaline the changes in activities were similar though not quite as great. This effect was reversible since similar measurements made 10 min after adrenaline addition (Table 1), when mechanical performance of the heart was back to normal, showed both activities had returned to their control values.

**Effect of cAMP-dependent protein kinase and phosphatase conditions on TGL and GPAT activities**

In rat adipose tissue, adrenaline has been shown to activate TGL via a cAMP-mediated, reversible phosphorylation system ("the lipolytic activation cascade") [54, 55]. GPAT activity is reduced when adipocytes are exposed to adrenaline [57, 52], possibly by a similar cAMP-mediated phosphorylation mechanism [43]. If the adrenaline effects reported in the previous section are similar to those seen in adipose tissue it might be expected that cAMP-dependent protein kinase would have an effect on these enzymes.

To test this, homogenates and supernatants from hearts perfused in the absence of adrenalin for 20 min were treated for 15 min with cAMP-dependent protein kinase together with ATP and cAMP (see methods). The activities of these homogenates with respect to GPAT and supernatants with respect to TGL were then measured. The results are shown in Table 2. With "cAMP-dependent" protein kinase, cAMP, and ATP present, TGL activity increased to 0.22 ± 0.04 above a control value of 0.17 ± 0.03. GPAT activity was reduced under the same conditions from 0.58 ± 0.01 to 0.28 ± 0.03. Similar results were obtained by using the catalytic subunit of cAMP-dependent protein kinase (prepared from the holoenzyme using a DE 52 ion exchange column and eluting with 20 µM cAMP) and ATP (results not shown). No changes were seen in the absence of added protein kinase, in the absence of ATP or in the absence of cAMP.

When using tissue from hearts perfused with adrenaline (Table 3) no activation of TGL activity by cAMP-dependent protein kinase above the already activated level was seen. Similarly GPAT activity was not changed from the low level seen in tissue from adrenaline-perfused hearts by incubation with cAMP-dependent protein kinase. However, after incubation in dephosphorylation conditions (endogenous phosphatase being stimulated by Ca^{2+} and Mg^{2+} ions) the activated
TABLE 2. The effect of cAMP-dependent protein kinase on the activity of glycerol 3-phosphate acyl transferase (GPAT) and triglyceride lipase (TGL) from rat heart homogenate and supernatant respectively

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Enzyme activity after 15 mins (nmol/min/mg)</th>
<th>GPAT</th>
<th>TGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer alone</td>
<td>0.59 ± 0.01</td>
<td>0.17 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Buffer + ATP</td>
<td>0.59 ± 0.03</td>
<td>0.17 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Buffer + cAMP + ATP</td>
<td>0.58 ± 0.03</td>
<td>0.15 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Buffer + cAMP + cAMP-dependent protein kinase</td>
<td>0.58 ± 0.03</td>
<td>0.16 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>cAMP + ATP + cAMP-dependent protein kinase</td>
<td>0.28 ± 0.03</td>
<td>0.22 ± 0.04²</td>
<td></td>
</tr>
</tbody>
</table>

Number of experiments = 3 (separate control perfused hearts).
Values are means ± s.e.m. Statistical significance = controls v. 1, P < 0.01 controls v. 2, P = 0.05.
Incubations for 15 min at 30°C as detailed in the methods section.

TGL activity and the inactivated GPAT activity produced by adrenalin perfusion had returned to the activities measured in control hearts (Table 3).

These results are consistent with those found for the same enzyme activities in adipose tissue and suggest that at least some of the effects produced by adrenalin could be mediated by cAMP-dependent protein kinase phosphorylations.

TABLE 3. The effect of cAMP-dependent protein kinase and dephosphorylation conditions on the activity of glycerol 3-phosphate acyl transferase (GPAT) and triglyceride lipase (TGL) in rat hearts perfused with adrenalin

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Enzyme activity (nmol/min/mg protein)</th>
<th>GPAT</th>
<th>TGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylation controls (Table 2)</td>
<td>0.30 ± 0.02</td>
<td>0.35 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>cAMP + ATP + cAMP-dependent protein kinase</td>
<td>0.29 ± 0.02</td>
<td>0.37 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Dephosphorylation control (without Ca²⁺, Mg²⁺)</td>
<td>0.32 ± 0.02¹</td>
<td>0.31 ± 0.04³</td>
<td></td>
</tr>
<tr>
<td>Dephosphorylation conditions (with Ca²⁺, Mg²⁺)</td>
<td>0.55 ± 0.07²</td>
<td>0.19 ± 0.08⁴</td>
<td></td>
</tr>
</tbody>
</table>

Number of experiments = 3 (separate adrenalin perfused hearts).
Values are means ± S.E.M.
Statistical significance: 1 v. 2, P < 0.03; 3 v. 4, P = 0.05.
Incubations for 15 min (phosphorylation) or 30 min (dephosphorylation) at 30°C as detailed in the methods section.
Adrenergic Control of GPAT and TGL Activities

The results of the previous experiment indicate that the observed changes in the activity of GPAT and TGL produced by adrenalin infusion could be brought about by activation of the \( \beta \)-adrenergic receptor leading to increased cAMP levels in the tissue. However, it has been shown that the rat myocardium contains not only \( \beta \)-adrenergic receptors \([1, 29]\) but \( \alpha_1 \) and \( \alpha_2 \) adrenergic receptors also \([3, 67]\). \( \alpha_1 \) receptors seem to predominate over \( \alpha_2 \) receptors \([67]\). It was decided to investigate the effect of specific \( \beta \), \( \alpha_1 \), \( \alpha_2 \) adrenergic stimulation of the activity of GPAT and TGL.

Table 4 shows the effect of perfusing the isolated rat heart with the \( \beta \)-agonist isoprenaline and the \( \alpha \)-agonist atenolol. Isoprenaline has been shown to increase the protein kinase activity ratio in heart tissue \([37]\). Our results show that at the time of peak mechanical effect the \( \beta \)-agonist decreased GPAT activity to a value similar to that found with adrenalin. TGL activity was increased by 100%. This is greater than the activation seen with adrenalin.

Continuous perfusion with the \( \alpha \)-agonist atenolol caused a small decline in both chronotropy and inotropy and a marked reduction in the activity of TGL. This suggests that even after extensive perfusion the control hearts are still expressing a degree of adrenergic stimulation. Thus the activity of TGL was seen to increase almost four-fold from a level of 0.13 ± 0.01 under \( \beta \)-adrenergic antagonism to 0.42 ± 0.07 with \( \beta \)-adrenergic stimulation.

The extensive reduction on GPAT activity seen with atenolol is surprising and suggests that the reductions in activity of this enzyme seen with both adrenalin and isoprenaline may not be solely due to cAMP-dependent protein kinase effects.

The effect of perfusion with \( \alpha \)-adrenergic agonists on the activity of GPAT and TGL is shown in Table 3. Methoxamine (a specific \( \alpha_1 \)-agonist) caused a small increase in inotropy concurrent with a rise in the resting tension, while a small increase in inotropy was rarely seen on perfusion with clonidine (a specific \( \alpha_2 \)-agonist). No change in chronotropy was measured in either case.

At a concentration of \( 10^{-6} \) M methoxamine produced no effect on the activity of TGL but decreased the activity of GPAT. The perfusion of clonidine at \( 10^{-5} \) M reduced the activity of TGL and increased the activity of GPAT. Smaller changes were seen at a concentration \( 10^{-6} \) M but these were not maximal and did not reach statistical significance. This result is similar to the \( \alpha_2 \)-adrenergic inhibition of lipolysis in other mammalian tissues \([15, 55]\), and is consistent, with a reduction in cAMP levels.

Continuous perfusion with the \( \alpha_1 \)-agonist doxazosin (Pfizer Central Research) and \( \alpha_2 \)-agonist yohimbine produced no change in the mechanics of the perfused heart or any changes in the activity of TGL and GPAT (results not shown).

Effect of adrenergic agents on microsomal and mitochondrial GPAT

Since there is general agreement that the principal site of triglyceride synthesis is the endo-

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**Table 4. The effect of isoprenaline infusion (10^{-6} M) and continuous perfusion with atenolol (10^{-6} M) on the activity of glycerol 3-phosphate acyl transferase (GPAT) and triglyceride lipase (TGL).**

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>GPAT (nmol/min/mg protein)</th>
<th>TGL (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.63 ± 0.07</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>0.31 ± 0.05^1</td>
<td>0.42 ± 0.07^2</td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.38 ± 0.08^2</td>
<td>0.13 ± 0.01^4</td>
</tr>
</tbody>
</table>

Values are expressed as (mean ± S.E.).

Statistical significance: controls v. 1 and 4 \( P < 0.001 \); controls v. 2 and 3 \( P < 0.01 \).
TABLE 3. The effect of infusion of the alpha, agonist methoxamine (10⁻⁶ M) and the alpha₂ agonist clonidine (10⁻⁴ M) on the activity of glycerol 3-phosphate acyl transferase (GPAT) and triglyceride lipase (TGL).

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>GPAT (nmol/min/mg protein)</th>
<th>TGL (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>[6] 0.63 ± 0.04</td>
<td>[6] 0.22 ± 0.02</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>[6] 0.49 ± 0.04</td>
<td>[6] 0.20 ± 0.03</td>
</tr>
<tr>
<td>Clonidine</td>
<td>[6] 0.79 ± 0.05</td>
<td>[6] 0.18 ± 0.01</td>
</tr>
</tbody>
</table>

Values are (Nos. of observations: mean ± s.e.). Statistical significance = control v. 1, P < 0.01. control v. 2, P < 0.01. control v. 3, P = 0.03.

TABLE 6. The response of NEM-sensitive (microsomal) and NEM-insensitive (mitochondrial) Glycerol 3-phosphate acyl transferase (GPAT) activity to adrenergic challenge.

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>GPAT activity (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NEM-sens(microsomal)</td>
</tr>
<tr>
<td>Control</td>
<td>[6] 0.35 ± 0.04</td>
</tr>
<tr>
<td>Adrenaline (10⁻⁶ M)</td>
<td>[6] 0.16 ± 0.05</td>
</tr>
<tr>
<td>Isoprenaline (10⁻⁶ M)</td>
<td>[6] 0.17 ± 0.06</td>
</tr>
<tr>
<td>Atenolol (10⁻⁶ M)</td>
<td>[6] 0.20 ± 0.04</td>
</tr>
<tr>
<td>Methoxamine (10⁻⁶ M)</td>
<td>[6] 0.27 ± 0.03</td>
</tr>
<tr>
<td>Clonidine (10⁻⁴ M)</td>
<td>[6] 0.50 ± 0.02</td>
</tr>
</tbody>
</table>

Values are (Nos. of observations: mean ± s.e.). Rat heart homogenate was incubated at 30°C for 5 min in the presence of either 15 mm N-ethylmaleimide (NEM) or sucrose buffer, and then assayed for GPAT activity.

Statistical significance = control v. 1, P < 0.01; v. 2, 3, 4 and 6, P < 0.05; v. 5, P = 0.001. All other differences are not significant.

Plasminic reticulum, attention has been focused, in other tissues, on the activity of the microsomal GPAT [5]. Rider and Saggerson [45], however, found both microsomal and mitochondrial forms in rat adipocytes to respond to noradrenaline. No information is available about such effects in the heart and it was decided to examine this. It is possible to distinguish between the two types by inhibiting the microsomal form with N-ethylmaleimide (NEM) [2, 3]. Table 6 shows the results of an investigation into the effects of the adrenergic challenge on the NEM-sensitive (microsomal) and NEM-insensitive (mitochondrial) GPAT activity.

Although adrenalin perfusion reduced both microsomal and mitochondrial forms of the enzyme the largest fall occurred in the microsomal form. With isoprenaline, atenolol, methoxamine and clonidine the changes seen in total GPAT activity appeared to be due largely to changes in the microsomal form.

Discussion

A considerable amount of evidence has accumulated to show the existence, in the heart, of lipases necessary for the hydrolysis of endogenous triglyceride known to be stored at intracellular sites [10, 26]. The heart also contains a substantial activity of lipoprotein lipase which will, under appropriate physiological conditions, hydrolyze circulating triglyceride before entry into the cardiac cell [46]. The
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contribution of lipoprotein lipase to the activity measured as triglyceride lipase in this investigation, was reduced to a minimum by using a phosphate buffer (pH 7.5) and avoiding any conditions known to increase the activity of lipoprotein lipase [47]. Also, by administering heparin in the intraperitoneal injection before the heart was excised, a large proportion of the extracellular lipoprotein lipase would have been removed [17].

Physiological evidence has appeared which indicates that intracellular triglyceride stores are important for myocardial function [21, 40], and that hormonal regulation of intracellular lipolysis occurs [28]. Catecholamines have been shown to increase the rate of triglyceride mobilization in perfused rat hearts [17, 22, 26, 31] and this has been characterized as a β-adrenergic mediated response [17, 31]. It is generally accepted that most of the β-adrenergic actions of catecholamines are mediated through increased levels of cAMP and enhanced protein kinase-catalyzed phosphorylations of key rate-limiting enzymes. Perfusion of rat hearts with catecholamines produced a rise in cAMP levels prior to increased glycerol release [17] and dibutylryl cAMP has been shown to decrease tissue levels of endogenous triglyceride [26]. Also Jesmok et al. [30] have demonstrated that increased rates of glycerol release from rat hearts perfused with catecholamines were associated with an increased activity ratio for protein kinase.

Our research supports the idea of a catecholamine-sensitive triglyceride lipase, responsive to the level of cAMP within the cardiac cell. We have shown that the β-agonist isoprenaline causes a marked increase in the activity of triglyceride lipase compared to hearts perfused with Krebs/Henseleit buffer alone. The incubation of fresh supernatant with cAMP-dependant protein kinase causes a small, but significant rise in TGL activity. Also stimulation of endogenous phosphatase decreases the adrenalin-activated activity to control values. Severson [47] could not detect any change in TGL activity in the presence of cAMP-dependant protein kinase. This was possibly due to its use of freeze-clamped tissue. In our hands TGL activity measured in supernatant from frozen tissue, was not activated by cAMP dependent protein kinase.

Continuous perfusion with the β-antagonist Atenolol reduced TGL activity to almost half that found in control hearts suggesting that the control hearts were exhibiting some degree of adrenergic stimulation, even after 15 min perfusion. This could arise by local release of endogenous noradrenalin from sympathetic nerve endings during the perfusion.

Concurrent with the increase in TGL activity seen under adrenalin stimulation, a fall in the activity of glycerol 3-phosphate acyl transferase (GPAT) was seen. This result is similar to that found by Saggerson and Sooranna [51, 32] with rat adipocytes. Incubation of fresh homogenate with cAMP-dependant protein kinase caused a marked decrease in GPAT activity over control values, while stimulation of endogenous phosphatase increases the adrenalin-deactivated activity, in agreement with the results reported by Nimmo and Houston [43] in adipose tissue. The fall in GPAT activity produced by isoprenaline could also be mediated by an increase in cAMP levels.

The combined effect of a rise in TGL activity and a fall in GPAT activity would be to cause a large increase in the availability of fatty acyl coA for subsequent oxidation to meet the increased energy demand made by a catecholamine-stimulated heart. Such reciprocal regulation of synthetic and degradative enzymes in opposing pathways is a common feature in metabolic control, but has not been reported previously for triglyceride mobilization in the heart.

Our results showing a fall in GPAT activity under β-antagonism, is anomalous. This suggests that the reduction in activity seen with this enzyme during adrenalin perfusion is not entirely due to β-adrenergic stimulation. This is at present under investigation.

Catecholamines not only interact with β-adrenergic receptors but also with those of the α-subtype. During the last several years both in vivo and in vitro experiments have demonstrated the presence of α-adrenergic receptors in the myocardium of a number of species [7]. Both, α1 and α2-adrenergic receptors have been shown to exist on the post-synaptic membrane of sympathetic nerve endings in the myocardium [35, 62]. Pre-synaptic α2-adrenergic receptors also exist and their
stimulation has been shown to reduce noradrenaline release from sympathetic nerves [23, 33]. x2-agonism, conversely, has been shown to potentiate noradrenaline release [23, 34].

The physiological significance of myocardial post-synaptic x2-adrenergic receptors has yet to be determined. In human fat cells [41], hamster adipocytes [13] and rat brown, but not white, adipose tissue [56]; an antilipolytic effect has been shown, caused by a fall in cAMP levels. This is due to x2-mediated reduction in the activity of membrane-bound adenylyl cyclase.

In our system, perfusion of the isolated rat heart with the x2-agonist clonidine resulted in a small fall in TGL activity but a significant rise in GPAT activity, results in agreement with a fall in cAMP levels. Thus stimulation of x2-mediated mechanisms could decrease cAMP levels either directly by decreasing the activity of membrane-bound adenylyl cyclase via post-synaptic x2-adrenergic receptors or indirectly by inhibiting the release of noradrenaline via pre-synaptic x2-adrenergic receptors.

Stimulation of the post-synaptic x2-adrenergic receptors increases the inotropic force of the heart [53, 60], possibly by increasing Ca2+ concentration in the cardiac cell [37]. This appears to be the result of an influx of calcium from extra- and/or intracellular storage sites [7, 6]. It has been proposed [36] that stimulation of phosphatidylinositol degradation is a primary and causal change involved in Ca2+ mobilization by x2-agonists. However, the mode of action is still not entirely clear [6, 36].

Perfusion of the isolated rat heart with the x2-agonists methoxamine and phenylephrine has been reported to increase glucose uptake, the phosphofructokinase activity ratio and lactate release [18, 19] suggesting enhanced glycolysis. The effect of x2-adrenergic stimulation on triglyceride mobilization has not been reported before. The present work has shown that perfusion of the isolated rat heart with the x2-agonist methoxamine, had no effect on TGL activity but produced a fall in GPAT activity. This effect has not been reported before and suggests that GPAT is sensitive to Ca2+ or some other x2-mediated signal.

Most of the changes reported in the activity of GPAT were shown to be localized in the NEM-sensitive or microsomal form of the enzyme. This is consistent with the generally held belief that the endoplasmic reticulum is the principal site of triglyceride synthesis.

Together these results provide evidence for the coordinated control of lipolysis and esterification in the heart by catecholamines, through the activity of glycerol 3-phosphate acyl transferase and triglyceride lipase. Both x- and x2-adrenergic mechanisms seem to be capable of altering the activity of these two enzymes. Their relative importance under normal and abnormal conditions remains to be resolved.

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