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THE EFFECTS OF NOREPINEPHRINE AND PROPRANOLOL ON MYOCARDIAL SUBCELLULAR DISTRIBUTION OF TRIGLYCERIDES AND FREE FATTY ACIDS[1,2,3]

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ABSTRACT

MASTERS, T. N. AND V. V. GLAVIANO: The effects of norepinephrine and propranolol on myocardial subcellular distribution of triglycerides and free fatty acids. J. Pharmacol. Exp. Ther. **182:** 246–255, 1972.

Myocardial subcellular alterations in free fatty acids (FFA) and triglycerides (TGFA) were measured in left ventricular muscle from open-chest mongrel dogs after the administration of propranolol (1 mg/kg i.v.) and norepinephrine (NE, 0.2 μg/kg/min i.v.). In one group of experiments (group I), FFA and TGFA levels were determined in nuclear, mitochondrial, microsomal and supernatant fractions in control dogs and in dogs after the administration of NE or propranolol. In this group of experiments, propranolol elevated the TGFA concentration in mitochondrial, microsomal and supernatant fractions from control whereas the levels of FFA in the same fractions declined, with the most significant changes occurring in the supernatant fraction. These results confirmed earlier reports from this laboratoy that propranolol may block myocardial FFA uptake by inhibiting intracellular TGFA degradation. NE was found to have no significant effect on the level (pool size) of FFA and TGFA in subcellular fractions of the myocardial muscle cell. In another group of experiments (group II), Na-palmitate-1-^{14}C (50 μc) was infused into the left circumflex coronary artery of dogs treated with propranolol or NE. Subcellular fractions were isolated and radioactivity of FFA, TGFA and phospholipids from these fractions was determined by liquid scintillation. Propranolol significantly increased activity of TGFA in the mitochondrial, microsomal and supernatant fractions which confirmed the inhibition of TGFA degradation found in group I with propranolol. However, NE increased the activity of TGFA in the mitochondrial, microsomal and supernatant fractions, indicating that the turnover of FFA through the TGFA

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moiety was increased, whereas its total intracellular level remained unchanged as observed in group I experiments. In response to both propranolol and NE, radioactivity in the FFA pool was reduced only in the supernatant fractions whereas little change was observed in phospholipid activity. The results of these experiments support the hypothesis that subcellular fractions containing pools of TGFA may have a role, especially in the supernatant fraction, in determining the myocardial uptake of FFA from arterial blood entering the heart.

Evans (1964) reported that free fatty acid (FFA) transport across the cardiac cell membrane is a passive process that depends on a concentration gradient resulting from intracellular utilization. A concentration gradient favoring the transport of FFA from blood to heart muscle can be iduced by elevating the arterial concentration (Bing, 1965). However, Opie (1968) reported that the transport of FFA involves more than just arterial levels since intracellular concentrations are approximately 20 times higher than those of blood. The higher concentration of intracellular FFA was postulated to result from intracellular "acceptors" or binding sites which physically bind FFA molecules prior to their activation to the acyl form (Evans, 1964). Removal of FFA from these binding sites by esterification or oxidation could influence indirectly membrane transport since FFA are incorporated into triglycerides (TGFA) prior to their intracellular oxidation (Shipp *et al.*, 1964). Drugs which could affect the size of the myocardial intracellular TGFA pool could in turn after myocardial FFA transport. Satchell *et al.* (1968) showed that propranolol increased the levels of TGFA in the isolated perfused heart whereas a decrease in TGFA was observed with norepinephrine (NE). Glaviano and Masters (1969) and Masters and Glaviano (1969) reported that, independent of changes in arterial FFA levels or cardiac hemodynamic parameters, FFA uptake by the heart is stimulated with NE and inhibited with the drug propranolol. Previous findings from this laboratory as well as those of Shipp *et al.* (1964) Satchell *et al.* (1968) would suggest that intracellular TGFA concentrations are involved in regulating FFA uptake by the myocardial cell. To seek evidence for this hypothesis, experiments were performed to determine the relationship beween FFA and TGFA in myocardial subcellular fractions of dogs treated with NE or propranolol. The results of these studies describe the distribution as well as the pool size of FFA and TGFA in subcellular fractions of the canine myocardium. In addition, the role that the intracellular TGFA pool may have in influencing the uptake of FFA was supported from the action that propranolol and NE have on the level and turnover of myocardial subcellular fractions of TGFA.

Methods

The changes resulting from the administration of NE or propranolol on myocardial subcellular lipid metabolism were determined in 32 mongrel dogs anesthetized by i.v. administration of sodium pentobarbital (30 mg/kg). The treachea was intubated from a midline neck incision and the vagosympathetic nerve trunks were isolated and sectioned at the level of the thyroid cartilage. To ensure adequate hydration, the right femoral vein was cannulated for a slow infusion of 100 to 150 ml of 0.9% NaCl solution. The chest was opened in the left 5th interspace while maintaining respiration with an Ensco model RU-4M respirator. The pericardium was incised and the heart was suspended in a pericardial cradle. The left femoral vein was cannulated for i.v. administration of drugs. The experiment was terminated by clamping the pulmonary artery and immediately removing the entire left ventricular myocardium. In one series of experiments (group I), alterations in myocardial subcellular FFA and TGFA concentrations were determined during the infusion of NE or after administration of the drug propranolol. The results from this group of experiments indicated changes in subcellular pool sizes of TGFA and FFA. In another series of experiments (group II), an indication of the rate at which arterial FFA were incorporated was determined from measuring the distribution of Na-palmitate-1-^{14}C in myocardial subcellular pools of FFA, TGFA and phospholipids under the same experimental conditions as in group I.

In group I experiments, control levels of FFA and TGFA were determined on cardiac muscle from six dogs, excised 10 minutes after thoracotomy. In 5 of 10 dogs, 10 minutes after the i.v. administration of propranolol (1 mg/kg), cardiac muscle samples were taken for analysis, whereas in the remaining 5 dogs, tissue was excised 10

minutes after initiating an i.v. infusion of 0.2 μg/kg/min of NE. The same dose of NE was used in dogs by Gold *et al.* (1965) and found to have a marked metabolic action with no significant effect on hemodynamics.

In group II experiments, the left circumflex coronary artery was cannulated close to its origin from the left common coronary artery. Blood was diverted from the right femoral artery with 3/16 inch (inside diameter) tygon tubing for perfusion of the coronary artery. Without interrupting coronary flow, Na-palmitate-1-^{14}C was infused into the heart from a PE 190 polyethylene catheter threaded from a T tube placed in the femoral-coronary artery perfusion circuit. The radioisotope was administered close to the femoral artery which permitted adequate mixing before entering the left circumflex coronary artery. One millicurie of Na-palmitate-1-^{14}C was prepared by the New England Nuclear Corporation (Boston, Mass.) with a specific activity of 8.0 mc/mmol. A stock solution was prepared by adding 10 ml of a 3.7% albumin-isotonic NaCl solution to the isotope and sonicating the mixture until complete emulsion had occurred. This represented a stock solution of 23:1 palmitate–albumin molar ratio, which was sealed and kept frozen at −20°C. One-half milliliter or 50 μc of stock solution was diluted with 4.5 ml of arterial blood collected from the experimental animal. The blood was mixed thoroughly with the isotope and infused with a Harvard infusion pump (model 975) at a rate of 2 ml/min with total infusion time of 2½ minutes. Control levels for this group (six dogs) were determined on cardiac muscle removed immediately after termination of the isotope infusion. In 5 of 10 dogs comprising group II, the isotope was infused 6½ minutes after 1 mg/kg of propranolol was administered. Tissue was excised after completion of the isotope infusion. In the remaining five dogs, the isotope infusion was started 7½ minutes after initiating an infusion of NE (0.2 μg/kg/min). The tissue was taken after completion of both infusions at 10 minutes.

After removal of the left ventricular myocardium in both groups I and II dogs, the tissue was cut into small pieces and placed immediately into a 100-ml beaker of ice-cold homogenizing media (0.32 M sucrose, 0.02 M Tris and 3×10^{-3} M $MgCl_2$ at pH 7.5). The mixture was maintained at 4°C and the homogenizing media was changed once after ½ hour. Superficial connective tissue, blood vessels and fat were removed in a 2°C cold room and discarded. After being cut into smaller pieces, the tissue was placed in a Harvard tissue press (model 141) and pressed through a fine attachment (1-mm diameter holes) to remove connective tissue. Approximately 18.1 to 18.3 g were weighed accurately and a 25% homogenate (1 part tissue, 3 parts homogenizing medium) was prepared with a Thomas tissue grinder (model 4288-B) immersed in ice. The homogenate was filtered by vacuum through six layers of 12-ply cheesecloth and the filtrate transferred into four 50-ml ultracentrifuge tubes (polycarbonate "Oak Ridge" type, approximately 14.5 ml per tube). To the filtrate in each tube were added 7.5 ml of 0.25 M sucrose and, after thorough mixing, 20 ml of the homogenizing medium were layered below the mixture prior to ultracentrifugation.

Subcellular fractions were isolated by differential ultracentrifugation (Schneider and Hogeboom, 1950) at 2°C in an International B-35 preparative ultracentrifuge with a fixed angle rotor (model A-147). A scheme outlining the centrifugation procedure is given in figure 1.

The pellets containing subcellular particles from the nuclear, mitochondrial and microsomal fractions were resuspended in 2 ml of 0.25 M sucrose by sonication. The supernatant fraction was pooled in a 250-ml graduate cylinder and thoroughly mixed and the volume was recorded. Precautions were taken to maintain the temperatures between 2–4°C prior to lipid extraction.

Lipids were extracted from the resuspended nuclear, mitochondrial and microsomal fractions and from 20 ml of the supernatant fraction according to the method of Dole and Meinertz (1960) as modified by Goss and Lein (1967). The upper heptane phase from each fraction was collected and reduced in volume to approximately 200 μl by directing a stream of N_2 over each sample. Individual lipid classes were separated by thin-layer chromatography on activated plates of Absorbosil-5 (Applied Science Laboratories, State College, Pa.). The plates were developed for 25 minutes with a mixture of petroleum ether (85 parts), diethyl ether (15 parts) and glacial acetic acid (1 part). In the concentration studies, FFA and TGFA were identified under ultraviolet light after spraying the plates with 2′,7′-dichlorofluorescein. In the radioisotope studies, identification was made by placing the plates in iodine vapors which were allowed to fade before scraping areas representing FFA, TGFA and phospholipids. Chloroform was used to elute both FFA and TGFA from the absorbant whereas anhydrous methanol was used to elute phospholipids.

FFA concentrations in the subcellular fractions were determined by directly titrating the eluate with Na-ethoxide and phenolphthalein as the indicator (Goss and Lein, 1967). Two 3-ml samples were titrated after washing the chloroform eluate five times with 0.02 N H_2SO_4. TGFA concentrations were determined by titrating the fatty acid moiety after saponification in ethanol KOH

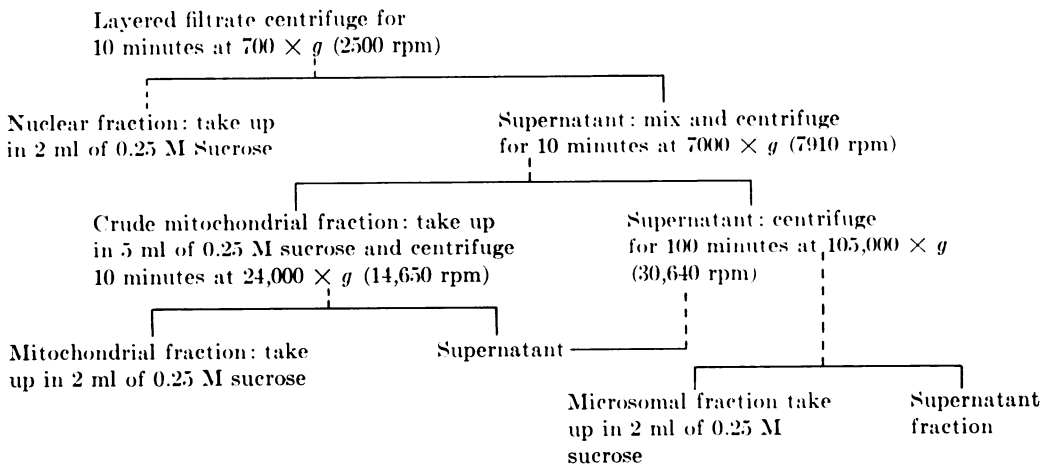

FIG. 1. Ultracentrifugation procedure for isolating subcellular fractions from dog heart homogenates.

(Sheath, 1965). Subcellular concentrations of FFA and TGFA were expressed in microequivalents of subcellular lipid per 100 g of left ventricular cardiac muscle. In the radioisotope studies, the eluate was collected directly in scintillation vials and evaporated to dryness by passing a N_2 stream over the chloroform or methanol containing the lipid. Ten milliliters of a cocktail containing 4 g of 2,5-diphenyloxazole and 5 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene per liter, freshly prepared with sulfur-free toluene, were added to each vial. The samples were counted in a Beckman LS-250 scintillation counter. Since there were differences in radioisotope uptake in individual animals as well as between the groups, the data was expressed as percentage of total subcellular activity. In this way proportional changes in activity of FFA, TGFA and phospholipids could be shown irrespective of the total radioactivity removed by the heart.

Statistical significance of metabolic changes observed was determined by Student's t test by unpaired analysis of changes between the control and propranolol dogs and between control and NE dogs in both group I (pool size) and group II (radioisotope) experiments.

Results

Figure 2 shows that i.v. administration of NE produced no significant change in the average concentration of FFA in the nuclear, mitochondrial, microsomal or supernatant fractions of cardiac muscle. The same figure shows that propranolol caused a significant reduction in levels of FFA in nuclear (6.98 to 2.51 μEq/g; $P < .05$), mitochondrial (11.04 to 1.85 μEq/g; $P < .05$), microsomal (11.50 to 1.15 μEq/g; $P < .05$) and supernatant (118.12 to 8.35 μEq/g; $P < .01$) fractions. On the other hand, the average concentrations of TGFA after administration of propranolol increased significantly in mitochondrial (16.7 to 33.04 μEq/g; $P < .05$), microsomal (24.27 to 42.51 μEq/g; $P < .05$), and supernatant (52.76 to 191.42 μEq/g; $P < .01$) fractions (fig. 3). After NE infusion, TGFA levels were not changed significantly, compared to control levels (fig. 3). The decline in subcellular levels of FFA (fig. 2) and the increase found in accompanying subcellular fractions of TGFA (fig. 3) would suggest that propranolol causes the intracellular TGFA pool to vary inversely with the pool size of FFA.

The postulated turnover of FFA through subcellular fractions of TGFA pools was determined by measuring Na-palmitate-1-^{14}C incorporation during the infusion of NE and after the administration of propranolol. The myocardial subcellular distribution of Na-palmitate-1-^{14}C during the infusion of NE or after propranolol (fig. 4) showed that the activity of FFA in the supernatant fraction was significantly reduced by both NE (35.9 to 28.8%; $P < .01$) and propranolol (35.9 to 27.3%; $P < .01$) whereas no significant changes were found in other subcellular fractions. On the other hand, although the levels of FFA in the different fractions were not altered by NE, NE increased the incorporation of Na-palmitate-1-^{14}C into TGFA in mitochondrial (2.8 to 5.6%; $P < .01$) and supernatant (4.5 to 12.2%; $P < .01$) fractions, as shown in figure 5. Although an increase was observed in the microsomal incorporation of the FFA into

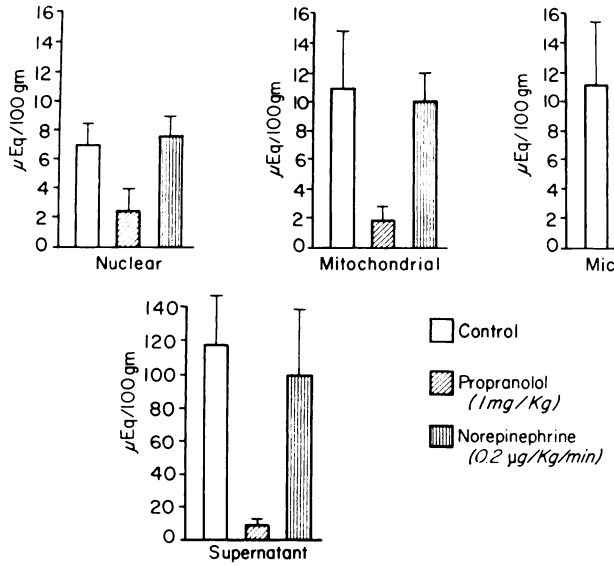

FIG. 2. A comparison of FFA concentrations in myocardial subcellular fractions between control dogs and dogs treated with propranolol or norepinephrine. Vertical lines on the bars represent the S.E.M.

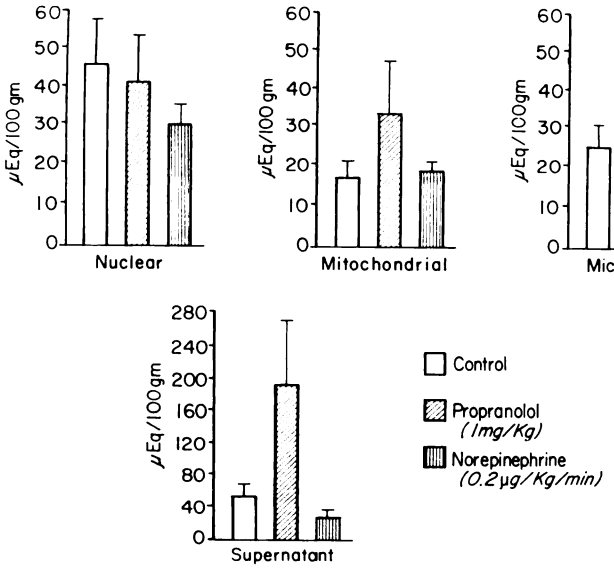

FIG. 3. Average changes in myocardial subcellular concentrations of TGFA in control dogs and in dogs during *beta* adrenergic receptor blockade or stimulation. Vertical lines on the bars represent the S.E.M.

TGFA, this increase was of borderline significance ($P = .05$). The action of propranolol, as graphed in figure 5, was found to be similar to NE, in that it also increased the incorporation of the labeled fatty acid in TGFA pools of mitocondrial (2.8 to 5.2%; $P < .05$), microsomal (6.6 to 9.6%; $P < .05$) and supernatant (5.4 to 9.5%; $P < .01$) fractions.

Although a reduction in activity of phospholipids can be noted in figure 6 to have occurred in all fractions after administration of propranolol, a significant change was found only

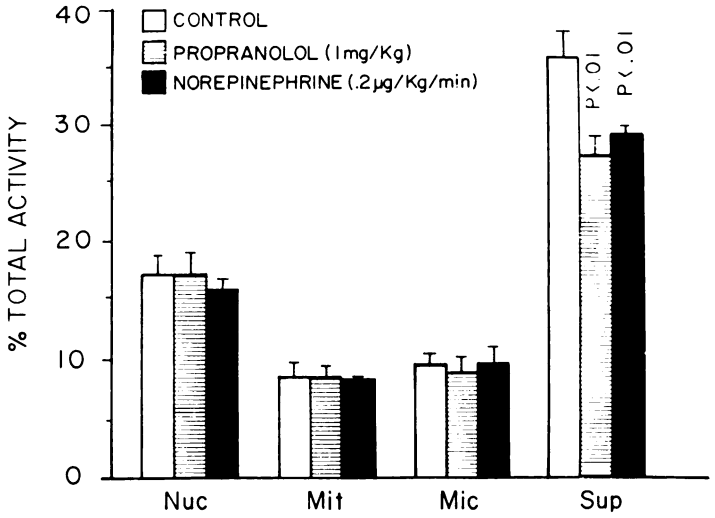

FIG. 4. Incorporation of Na-palmitate-1-^{14}C into myocardial FFA of nuclear (NuC), mitochondrial (MiT), microsomal (MiC) and supernatant (SuP) fractions in control dogs and in dogs treated with propranolol or norepinephrine. The results are expressed as a percentage of total activity and the S.E.M. is represented by the vertical line above each bar.

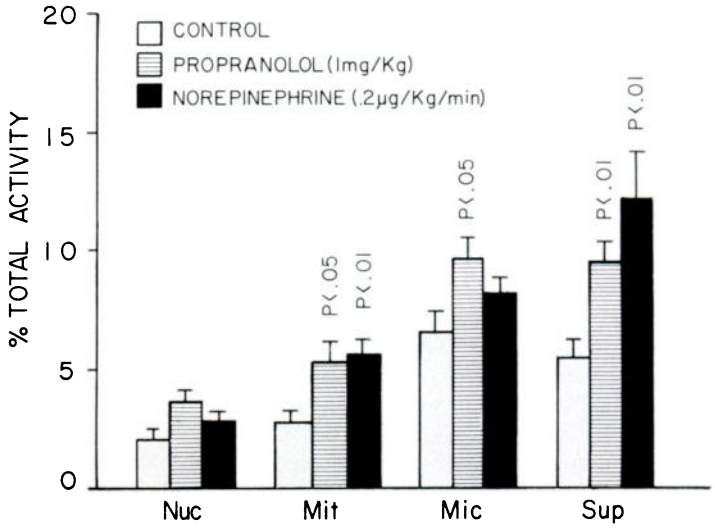

FIG. 5. Myocardial subcellular incorporation of label palmitate into myocardial TGFA of nuclear (NuC), mitochondrial (MiT), microsomal (MiC) and supernatant (SuP) fractions in control dogs and in dogs dogs subjected to *beta* adrenergic receptor blockade or stimulation. The results are expressed as a percentage of total activity and the S.E.M. is represented by the vertical line above each bar.

in the supernatant fraction. The infusion of NE had no significant effect on the incorporation of the labeled FFA.

Discussion

Work reported from other laboratories on cardiac metabolism has indicated that the heart plays a passive role in regulating FFA uptake. Coronary blood flow (Namm and Rosenblum, 1966; Cowley *et al.*, 1969) as well as arterial FFA levels (Bing, 1965), plasma albumin/FFA molar ratio, fatty acid chain length and degree of unsaturation (Evans, 1964) were found to alter the uptake of FFA by the heart. However,

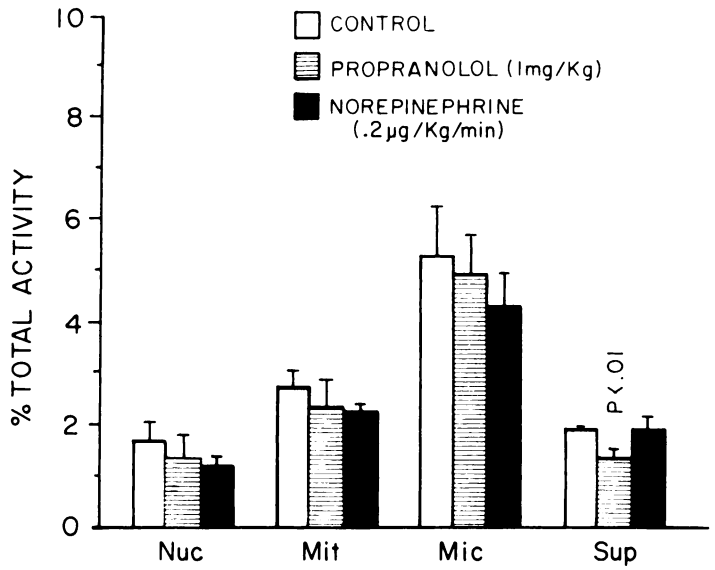

FIG. 6. The incorporation of labeled palmitate into myocardial phospholipids of nuclear (NuC), mitochondrial (MiT), microsomal (MiC) and supernatant (SuP) fractions in control dogs and in dogs treated with propranolol or norepinephrine. The results are expressed as a percentage of total activity and the S.E.M. is represented by the vertical line above each bar.

other laboratories have suggested that myocardial intracellular TGFA synthesis and degradation have an important role in the myocardial uptake and utilization of FFA. Shipp *et al.* (1964) reported that FFA taken up by the heart are first incorporated into TGFA prior to their intracellular utilization. Challoner and Steinberg (1966a,b) found that epinephrine increased the O_2 consumption of the K⁺-arrested isolated rat heart. The increase in O_2 consumption was found to be related to an accelerated degradation of TGFA, giving rise to an elevated intracellular level of FFA. Therefore, it would seem from previous reports that the rate of intracellular TGFA degradation may be associated with the rate of uptake of FFA by the heart. If TGFA degradation were stimulated, greater intracellular availability of FFA would result as well as an increase in potential sites for esterification of transported FFA.

The results of the reported experiments implicate a possible role for the myocardial adenyl cyclase system in the transport of myocardial FFA since both propranolol and NE may exert their action, at least in part, on altering levels of cyclic adenosine 3′,5′-monophosphate (c-AMP) (Skelton *et al.*, 1970; Peterson *et al.*, 1968). The mechanism through which the adenyl cyclase system could influence myocardial FFA uptake may be similar to the mechanism which mobilizes FFA from adipose tissue (Rizack, 1965; Sutherland *et al.*, 1968). The substrates and enzymes necessary for FFA release from adipose tissue also have been found in cardiac muscle. These include the adenyl cyclase system (Murad *et al.*, 1962), a hormone-sensitive lipase (Björntorp and Furman, 1962) and a TGFA pool that serves as a source for FFA (Scheuer and Olson, 1967). It has been found in adipose tissue that drugs inhibiting the formation of c-AMP prevented the release of FFA and greatly increased TGFA concentrations (Sutherland *et al.*, 1968). Similarly, in cardiac muscle, Satchell *et al.* (1968) reported that propranolol in the isolated heart elevated endogenous TGFA levels. In the *in situ* heart, intracoronary administration of prostaglandin E_1 increased TGFA in heart muscle while decreasing myocardial uptake of FFA (Glaviano and Masters, 1971). The antilipolytic action of prostaglandin E_1 on the heart was found to be due to a decline in levels of c-AMP. On the other hand, stimulation of FFA mobilization from intracellular TGFA was reported in the isolated rat heart by Gousios *et al.* (1965) and Kreisberg (1966). They showed that epinephrine increased utilization of endogenous TGFA, whereas in the *in situ* heart, NE increased myocardial FFA uptake (Gold *et al.*, 1965; Cowley *et al.*, 1969; Glaviano and Masters, 1969). The changes produced by NE in FFA uptake by the heart would

suggest that a close relationship is likely between synthesis and degradation of TGFA and myocardial uptake of FFA.

In the reported experiments, propranolol elevated the pool size of TGFA in mitochondrial, microsomal and supernatant fractions (fig. 3) while reducing the level of FFA in the same fractions (fig. 2). The increase in concentration of TGFA in these subcellular compartments with propranolol also was confirmed in the radioisotope studies in which a greater incorporation of Na-palmitate-1-^{14}C into TGFA was found, with the exception of the nuclear fraction, in all subcellular fractions (fig. 5). These findings would imply that propranolol causes an increased in intracellular TGFA concentration by inhibiting its degradation, an observation that supports the decrease found in the myocardial pool of FFA in different subcellular fractions (fig. 2). A concomitant decrease in Na-palmitate-1-^{14}C incorporation into the FFA pool with propranolol was observed only in the supernatant fraction, which could reflect a decrease in available unlabeled FFA for exchange with the isotope. A decline similar to that found in the supernatant fraction for the labeled FFA was expected to also occur in the microsomal and mitochondrial fractions. However, if one accepts adenyl cyclase to be located primarily at or in the plasma membrane (Rosen and Rosen, 1969; Pohl *et al.*, 1969), then drugs stimulating or inhibiting adenyl cyclase could exert their action only on the supernatant fraction.

That TGFA synthesis is being stimulated by NE can be noted from the increase in incorporation of the labeled FFA into the TGFA moiety in the supernatant fraction ($P < .01$; fig. 5). This increase in rate of incorporation of the isotope into the TGFA pool by NE was also observed for the mitochondrial fraction ($P < .01$) and for the microsomal fraction which was of borderline significance ($P = .05$).

Norepinephrine produced no significant change in subcellular concentration of TGFA from control (fig. 3). If TGFA degradation is related to the transport of FFA into the cell, the lack of change in overall concentration of TGFA may have reflected an increase in turnover of FFA molecules through the TGFA moiety. This action of NE was supported by the significant increase in incorporation of the percent total activity of TGFA in microsomal, mitochondrial and supernatant fractions (fig. 5). The rise observed in the rate of FFA incorporated during the NE infusion would suggest that the labeled fatty acid extracted by the heart was exchanged with preexisting unlabeled triglyceride fatty acids in the mitochondrial, microsomal and supernatant fraction at a faster rate without affecting a change in the size of TGFA pool. As previously noted the pool size of TGFA in the different subcellular fractions was not observed to change during the infusion of NE (fig. 3).

The rate of TGFA degradation represents a plausible mechanism for influencing the uptake of FFA by the heart. The scheme given in figure 7 illustrates a cytoplasmic location of this mechanism which is supported by the observed changes occurring primarily in the supernatant fraction. The requirements for such a mechanism

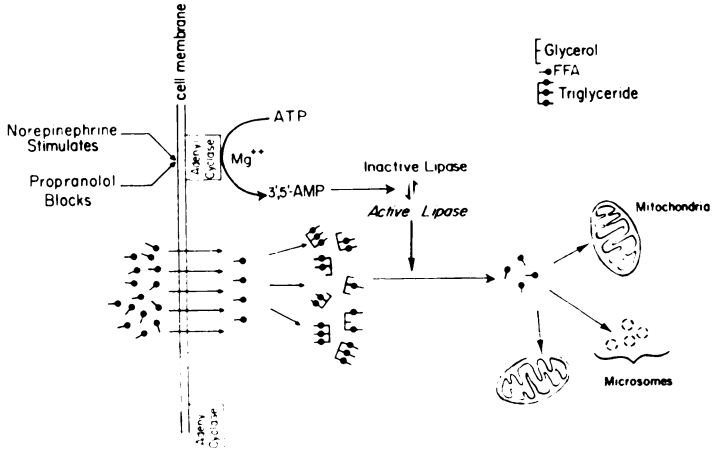

FIG. 7. Diagrammatic representation of an intracellular mechanism postulated for regulating the myocardial uptake of FFA.

to operate would be that 1) FFA taken up by the heart first are incorporated into TGFA before their intracellular utilization (Shipp *et al.*, 1964), 2) it requires the adenyl cyclase system for the production of c-AMP (Sutherland *et al.*, 1962; Murad *et al.*, 1962; Robison *et al.*, 1965), 3) an intracellular lipase that is responsive to changes in c-AMP levels (Kruger *et al.*, 1967) which in turn can be stimulated in the heart by catecholamines (Björntorp and Furman, 1962; Robison *et al.*, 1965; Namm and Mayer, 1968) and 4) a fairly stable pool of cytoplasmic glycerol molecules which could serve as an intracellular acceptor for FFA molecules (Scheuer and Olson, 1967). These reports in conjunction with the present studies suggest that propranolol could inhibit myocardial FFA uptake by causing a decrease in the synthesis of intracellular c-AMP levels (Robison *et al.*, 1967). The decrease in lipase activity resulting from decline in levels of c-AMP would prevent TGFA degradation (Okamoto *et al.*, 1971) which in turn decreases FFA uptake by the heart. However, an increase in adenyl cyclase activity, such as the response to NE, causes an elevation in c-AMP levels which in turn can increase intracellular triglyceride lipase activity.

A preliminary report from this laboratory (Masters and Glaviano, 1970) indicated that dibutyryl c-AMP was found to reverse, in dogs, the inhibition of myocardial FFA uptake produced after the administration of propranolol. The blockade to FFA uptake was reversed with dibutyryl c-AMP without increasing cardiac contractility or FFA arterial levels. It was concluded from this study that dibutyryl c-AMP reversed myocardial lipogenesis by activating the intracellular lipase that causes TGFA degradation.

The reported experiments indicate that FFA transport across the cardiac cell membrane is influenced by intracellular mechanisms that affect the synthesis and degradation of TGFA.

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References

Bing, R. J.: Cardiac metabolism. Physiol. Rev. **45**: 171–213, 1965.

Björntorp, P. and Furman, R. H.: Lipolytic activity in rat heart. Amer. J. Physiol. **203**: 323–326, 1962.

Challoner, D. R. and Steinberg, D.: Effect of free fatty acid on the oxygen consumption of perfused rat heart. Amer. J. Physiol. **210**: 280–286, 1966a.

Challoner, D. R. and Steinberg, D.: Oxidative metabolism of myocardium as influenced by fatty acids and epinephrine. Amer. J. Physiol. **211**: 897–902, 1966b.

Cowley, A. W., Scott, J. C. and Spitzer, J. J.: Myocardial FFA metabolism during coronary infusion of norepinephrine in conscious dogs. Amer. J. Physiol. **217**: 511–517, 1969.

Dole, V. P. and Meinertz, H.: Microdetermination of long-chain fatty acids in plasma and tissues. J. Biol. Chem. **235**: 2595–2599, 1960.

Evans, J. R.: Cellular transport of long chain fatty acids. Can. J. Biochem. **42**: 955–969, 1964.

Glaviano, V. V. and Masters, T. N.: Effect of norepinephrine on myocardial metabolism before and after beta receptor blockade. Eur. J. Pharmacol. **7**: 135–142, 1969.

Glaviano, V. V. and Masters, T. N.: Inhibitory action of intracoronary prostaglandin E_1 on myocardial lipolysis. Amer. J. Physiol. **220**: 1187–1193, 1971.

Gold, M., Houshang, J. A., Spitzer, J. J. and Scott, J. C.: Effect of norepinephrine on myocardial free fatty acid uptake and oxidation. Proc. Soc. Exp. Biol. Med. **118**: 876–879, 1965.

Goss, J. E. and Lein, A.: Microtitration of free fatty acids in plasma. Clin. Chem. **13**: 36–39, 1967.

Gousios, A., Felts, J. M. and Havel, B. J.: Effect of catecholamines, glucose, insulin and changes of flow on the metabolism of free fatty acids by the myocardium. Metabolism (Clin. Exp.) **14**: 826–831, 1965.

Kreisberg, R. A.: Effect of epinephrine on myocardial triglyceride and free fatty acid utilization. Amer. J. Physiol. **210**: 385–388, 1966.

Kruger, F. A., Leighty, E. G. and Weissler, A. M.: Catecholamine stimulation of myocardial lipolysis and fatty acid reesterification. J. Clin. Invest. **46**: 1080–1081, 1967.

Masters, T. N. and Glaviano, V. V.: Effect of *dl*-propranolol on myocardial free fatty acid and carbohydrate metabolism. J. Pharmacol. Exp. Ther. **167**: 187–193, 1969.

Masters, T. N. and Glaviano, V. V.: Myocardial subcellular distribution of Na-palmitate-1-^{14}C and the effects of dibutyryl c-AMP on β-adrenergic receptor activity and fatty acid metabolism Fed. Proc. **29**: 449, 1970.

Murad, F., Chi, Y. M., Rall, T. W. and Sutherland, E. W.: Adenyl cyclase. III. The effect of catecholamines and choline esters on the formation of adenosine 3′,5′-phosphate by preparations from cardiac muscle and liver. J. Biol. Chem. **237**: 1233–1238, 1962.

Namm, D. H. and Mayer, S. E.: Effects of epinephrine on cardiac cyclic 3′,5′-AMP, phosphorylase kinase, and phosphorylase. Mol. Pharmacol. **4**: 61–69, 1968.

Namm, D. H. and Rosenblum, I.: Effects of sympathomimetic amines on canine coronary arteriovenous differences of glucose and non-esterified fatty acids. Arch. Int. Pharmacodyn. Thér. **161**: 175–182, 1966.

OKAMOTO, R., GLAVIANO, V. V. AND PINDOK, M.: Myocardial lipases and catecholamines in burn shock. Proc. Soc. Exp. Biol. Med. **137**: 347–353, 1971.

OPIE, L. H.: Metabolism of the heart in health and disease. Part I. Amer. Heart J. **76**: 685–698, 1968.

PETERSON, M. J., PATTERSON, C. AND ASHMORE, J.: Effects of antilipolytic agents on dibutyryl cyclic AMP induced lipolysis in adipose tissue. Life. Sci. **7**: 551–560, 1968.

POHL, S. L., BIRNBAUMER, L. AND RODBELL, M.: Glucagon-sensitive adenyl cyclase in plasma membrane of hepatic parenchymal cells. Science (Washington) **164**: 566–567, 1969.

RIZACK, M. A.: Hormone-sensitive lipolytic activity of adipose tissue. *In* Handbook of Physiology, Section 5, Adipose Tissue, ed. by A. E. Renold and C. F. Cahill, Jr., pp. 309–311, American Physiological Society, Washington, D. C., 1965.

ROBISON, G. A., BUTCHER, R. W., ØYE, I., MORGAN, H. E. AND SUTHERLAND, E. W.: The effect of epinephrine on adenosine 3′,5′-phosphate levels in the isolated perfused rat heart. Mol. Pharmacol. **1**: 168–177, 1965.

ROBISON, G. A., BUTCHER, R. W. AND SUTHERLAND, E. W.: Adenyl cyclase as an adrenergic receptor. Ann. N.Y. Acad. Sci. **139**: 703–723, 1967.

ROSEN, O. M. AND ROSEN, S. M.: Properties of an adenyl cyclase partially purified from frog erythrocytes. Arch. Biochem. Biophys. **131**: 449–456, 1969.

SATCHELL, D. G., FREEMAN, S. E. AND EDWARDS, S. V.: Effects of beta-receptor blocking drugs on cardiac metabolism. Biochem. Pharmacol. **17**: 45–54, 1968.

SCHEUER, J. AND OLSON, R. E.: Metabolism of exogenous triglyceride by the isolated perfused rat heart. Amer. J. Physiol. **212**: 301–307, 1967.

SCHNEIDER, W. C. AND HOGEBOOM, G. H.: Intracellular distribution of enzymes, V. Further studies on the distribution of cytochrome c in rat liver homogenates. J. Biol. Chem. **183**: 123–128, 1950.

SHEATH, J.: Estimation of plasma non-esterified fatty acids and triglyceride fatty acids by thin-layer chromatography and colorimetry. Aust. J. Exp. Biol. Med. Sci. **43**: 563–572, 1965.

SHIPP, J. C., THOMAS, J. M. AND CREVASSE, L.: Oxidation of carbon-14-labelled endogenous lipids by isolated perfused rat hearts. Science (Washington) **143**: 371–373, 1964.

SKELTON, C. L., LEVY, G. S. AND EPSTEIN, S. E.: Positive inotropic effects of dibutyryl cyclic adenosine 3′,5′-monophosphate. Circ. Res. **26**: 35–43, 1970.

SUTHERLAND, E. W., RALL, T. W. AND MENNO, T.: Adenyl cyclase: I. Distribution, preparation and properties. J. Biol. Chem. **237**: 1220–1227, 1962.

SUTHERLAND, E. W., ROBISON, G. A. AND BUTCHER, R. W.: Some aspects of the biological role of adenosine 3′,5′-monophosphate (cyclic AMP). Circulation **37**: 279–306, 1968.