Enhancement of Fatty Acid Oxidation and Medium-Chain Fatty Acyl Coenzyme A Synthetase by Adenine Nucleotides in Rat Heart Homogenates

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Abstract ☐ Cyclic 3′,5′-adenosine monophosphate, 5′-adenosine monophosphate, or 2′-adenosine monophosphate markedly enhanced the rate of oxidation of medium-chain fatty acids by rat heart homogenates, as measured by oxygen utilization and carbon dioxide formation from ^{14}C-labeled substrate. These nucleotides did not alter the rate of oxidation of medium-chain acylcoenzyme A derivatives. The activity of a medium-chain fatty acyl coenzyme A synthetase from rat heart homogenates was increased by these nucleotides, and it was suggested that the ability of the nucleotides to enhance fatty acid oxidation by heart homogenates was due to activation of acyl coenzyme A synthetase.

Keyphrases ☐ Adenine nucleotides—rat heart homogenates ☐ Oxygen-uptake enhancement, rat heart homogenates—adenine nucleotides ☐ $^{14}CO_2$-evolution, 1-^{14}C-octanoate—adenine nucleotide enhancement ☐ Acyl coenzyme A synthetase—rat heart nucleotide effect ☐ Scintillometry—analysis

Recent observations suggest that various nucleotides, including 5′-adenosine monophosphate (5′-AMP) and cyclic 3′,5′-adenosine monophosphate (3′,5′-AMP), may be involved in the regulation of energy metabolism (1). The ability of 5′-AMP and 5′-adenosine diphosphate (5′-ADP) to enhance liver glutamate dehydrogenase activity has been well established (2, 3). In addition, these nucleotides have been found to alter the catalytic activity of isocitric dehydrogenase (4) and phosphofructokinase (5). Mansour (6) showed that 5′-AMP and cyclic 3′,5′-AMP activate phosphofructokinase isolated from guinea pig heart and from a parasitic liver fluke.

Studies by many investigators (7–16) indicated that cyclic 3′,5′-AMP, which is formed from adenosine triphosphate (ATP) by the enzyme adenyl cyclase, is involved in the regulation of various metabolic pathways including glycogenolysis, lipolysis, and steroidogenesis. Recently Harada *et al.* (17) reported that starvation of animals on alloxan treatment produced a marked increase in acylcoenzyme A synthetase activity. In addition, Bar-Tana and Rose (18, 19) observed heterotropic cooperative effects with 5′-ATP or 5′-AMP on a partially purified medium-chain acyl coenzyme A synthetase from bovine liver. Inasmuch as the heart can derive much of its required energy from fatty acid oxidation (20–22), the actions of various nucleotides, including cyclic 3′,5′-AMP, on the metabolism of fatty acids were investigated.

MATERIALS AND METHODS

All nucleotides[1] used in this study were found to be 99% pure when assayed spectrophotometrically. They were demonstrated to be homogeneous by a descending paper chromatographic technique with a solvent system consisting of 3 parts 1 *M* ammonium acetate (adjusted to pH 7.5 with ammonium hydroxide) and 7 parts 95% ethanol. Molar absorbances and R_f values were virtually identical to values reported previously (23).

Adult Sprague-Dawley rats of either sex were decapitated, and the hearts were homogenized in sufficient 0.25 *M* sucrose to make a 10% suspension. Homogenization was performed in a tight-fitting glass homogenizer by several passes of a Teflon pestle, driven at approximately 1200 r.p.m. The homogenate (0.5 ml.) was then added to Warburg flasks containing 0.1 m*M* ATP; 0.03 m*M* CoA; 0.03 m*M* $MgCl_2$; 0.3 m*M* nicotinamide; 1 m*M* theophylline; 0.1 m*M* carnitine; 4 m*M* phosphate buffer, pH 7.4; and 0.17 m*M*

Table I—Effects of Adenine Nucleotides on the Rates of Oxidation of Various Substances by Heart Homogenates

Substrate	Nucleotide	Q_{O_2}[a]	Percent Stimulation
Octanoate[b]	None	1.92 ± 0.21	—
Octanoate[b]	3′,5′-AMP	5.82 ± 0.40	203
Octanoate[b]	5′-AMP	5.97 ± 0.42	211
Octanoate[b]	2′-AMP	5.34 ± 0.39	178
Butyrate	None	2.08 ± 0.25	—
Butyrate	3′,5′-AMP	5.74 ± 0.38	176
Butyrate	5′-AMP	6.10 ± 0.42	193
Butyrate	2′-AMP	5.00 ± 0.40	140
β-Hydroxybutyrate	None	2.96 ± 0.29	—
β-Hydroxybutyrate	3′,5′-AMP	5.20 ± 0.38	75
β-Hydroxybutyrate	5′-AMP	6.89 ± 0.39	133
β-Hydroxybutyrate	2′-AMP	5.55 ± 0.35	87
Pyruvate	None	4.42 ± 0.26	—
Pyruvate	3′,5′-AMP	8.00 ± 0.37	81
Pyruvate	5′-AMP	8.30 ± 0.40	88
Pyruvate	2′-AMP	8.25 ± 0.37	86
α-Ketoglutarate	None	3.52 ± 0.30	—
α-Ketoglutarate	3′,5′-AMP	9.22 ± 0.41	162
α-Ketoglutarate	5′-AMP	9.46 ± 0.46	169
α-Ketoglutarate	2′-AMP	7.70 ± 0.39	119
Glutamate	None	3.98 ± 0.20	—
Glutamate	3′,5′-AMP	4.88 ± 0.40	22
Glutamate	5′-AMP	6.85 ± 0.36	72
Glutamate	2′-AMP	4.91 ± 0.30	23
Succinate	None	9.28 ± 0.31	—
Succinate	3′,5′-AMP	10.79 ± 0.21	16[c]
Succinate	5′-AMP	10.53 ± 0.35	13
Succinate	2′-AMP	10.01 ± 0.40	7
Malate	None	3.25 ± 0.19	—
Malate	3′,5′-AMP	3.45 ± 0.23	—
Malate	5′-AMP	3.18 ± 0.31	—
Malate	2′-AMP	3.85 ± 0.26	—
Citrate	None	2.44 ± 0.18	—
Citrate	3′,5′-AMP	2.12 ± 0.17	—
Citrate	5′-AMP	2.17 ± 0.22	—
Citrate	2′-AMP	2.00 ± 0.20	—

[a] Q_{O_2} values represent microliters of oxygen uptake per milligram protein per 22.5 min. at 37° and represent the average of four–five experiments with the standard error of the mean. Corrections were made for oxidation of endogenous substrates in the presence or absence of nucleotide. The average value from 10 separate experiments for the endogenous rate of oxygen uptake in the presence or absence of nucleotide was 1.30 μl./22.5 min. [b] Octanoate concentration was 0.17 m*M*, which was found to give optimal rates of oxygen uptake. [c] These results are not significant at the 95% probability level as shown by the Student *t* test. All other results are significant at this level.

[1] Obtained from Sigma Chemical Co., St. Louis, Mo.

Table II—Effect of Theophylline on Conversion of Cyclic 3′,5′-AMP to 5′-AMP by Heart Homogenates

Minutes	Theophylline, mM	5′-AMP, c.p.m.[a]	Cyclic 3′,5′-AMP, c.p.m.	% 3′,5′-AMP Hydrolyzed
15	0	269	4466	5.6
15	1.0	116	4857	2.3
30	0	585	4428	11.6
30	1.0	319	4340	6.8
45	0	900	4225	17.5
45	1.0	497	4353	10.2

[a] Cyclic 3′,5′-AMP (Schwartz) contained approximately 3% 5′-AMP (representing about 200 c.p.m. in these experiments), and the values in this column were appropriately corrected. Experimental details are given in the text.

nucleotide or water. The side arms contained substrate and malate (24). The total volume was 3.0 ml., and the pH of each added solution was 7.4. All reagents used were the highest quality available and were prepared in triple-distilled water. After an equilibration period of 10 min., the flasks were closed to the atmosphere, the substrate was tipped from the side arms, and oxygen uptake was measured at 7.5-min. intervals for 45 min. at 37° (25). All rates of oxygen uptake were linear for 22.5 min. Each determination of oxygen uptake was performed in triplicate and repeated at least three times.

Octanoyl coenzyme A and butyryl coenzyme A were prepared by the method of Stadtman (26). The reaction of butyric anhydride or octanoic anhydride with coenzyme A was conducted under an atmosphere of N_2, and completion of the reaction was determined by the nitroprusside test for free sulfhydryl groups. Octanoyl coenzyme A or butyryl coenzyme A was then separated from the reaction mixture on a 1 × 50-cm. synthetic polysaccharide[2] column. The concentration of the thioester was determined by the method of Lipmann and Tuttle (27) with sodium butyrohydroxamate as a standard. The yield of butyryl coenzyme A was 90% based on the amount of coenzyme A used in the reaction. The yield of octanoyl coenzyme A was slightly less than 90%. The preparations were not contaminated with free unoxidized coenzyme A, as indicated by an absence of color formation with nitroprusside or with Ellman (28) reagent [5,5′-dithiobis-(2-nitrobenzoic acid)].

Studies with 1-^{14}C-octanoate were performed in double side-arm Warburg flasks closed with glass stoppers. The main compartment of each flask contained the necessary reagents as previously described. At zero time, 0.1 ml. of 5.0 mM 1-^{14}C-octanoate (specific activity 0.57 mc./mmole) was tipped from the first side arm, and the flasks were allowed to shake gently for 45 min. at 37° in a Dubnoff metabolic shaker. The reaction was stopped by tipping H_2SO_4 from the second side arm of each flask, followed by a 30-min. incubation period to allow the $^{14}CO_2$ to be trapped in the center wells. The center wells contained 0.2 ml. of a mixture of 0.01 mmole NaOH and 1.5 mmoles piperidine. After incubation, aliquots were transferred to vials containing scintillation fluid, which consisted of 7 g. PPO and 0.42 g. POPOP/l. of toluene.[3] Determination of radioactivity was performed with a Packard liquid scintillation spectrometer, model 3310. In all studies, values for background radioactivity were obtained by using a boiled enzyme preparation; values reported in this paper were corrected for this activity.

The activity of a medium-chain fatty acyl coenzyme A synthetase from rat heart homogenates with butyrate or octanoate as substrates was determined by the method of Kornberg and Pricer (29), using hydroxylamine to trap the acylthioesters. The incubation medium was as described with 0.3 M hydroxylamine and 0.1 M tromethamine buffer, pH 8.0. All components were adjusted to pH 8.0 except coenzyme A. Reaction mixtures without fatty acid substrates or ATP served as controls, and the reactions were initiated by addition of homogenate. After incubation at 37° for 30 min., the reaction was stopped by addition of 1 ml. of a freshly prepared solution of 10% $FeCl_3$ in 0.2 N HCl with 7% perchloric acid. Following centrifugation to sediment protein, the optical density of the supernatant was

[2] Sephadex G-10, Pharmacia Fine Chemicals, Piscataway, N. J.
[3] PPO refers to 2,5-dimethylphenyloxazole, and POPOP represents 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene.

Table III—Enhancement of Octanoate Oxidation by Cyclic 3′,5′-AMP or 5′-AMP in Heart Homogenates Aged for Various Times at 0°

Minutes after Preparation of Homogenate	Nucleotide	Q_{O_2}[a]	Increase, %
10	None	1.73 ± 0.21	—
10	5′-AMP	5.70 ± 0.45	229
10	3′,5′-AMP	5.61 ± 0.39	224
20	None	0.35 ± 0.05	—
20	5′-AMP	2.93 ± 0.17	737
20	3′,5′-AMP	2.58 ± 0.20	637
30	None	0.11 ± 0.03	—
30	5′-AMP	2.82 ± 0.33	2463
30	3′,5′-AMP	2.73 ± 0.29	2381

[a] Experimental conditions were as described in Table I and the text. Corrections were made for endogenous oxygen uptake as in Table I, except Q_{O_2} values for the 10-, 20-, and 30-min. times were 1.27, 1.23, and 0.64 µl. oxygen uptake/22.5 min., respectively. Sufficient nucleotide was added to give final concentrations of 0.17 mM. Ten percent heart homogenates were aged at 0° for the times indicated before being added to the flasks. Q_{O_2} values represent microliters of oxygen uptake per milligram protein/22.5 min. at 37° and represent the average of four experiments with the standard deviation. Results are significant at the 95% probability level.

determined at 520 nm. All protein determinations were performed by the biuret procedure with bovine serum albumin as a standard.

The effect of theophylline on the conversion of cyclic 3′,5′-AMP to 5′-AMP by heart homogenates was determined by incubating 0.33 mM 8-^{14}C-cyclic 3′,5′-AMP (specific activity 1.0 µc./µmole) with a complete reaction mixture as already described. The reaction mixture was incubated for various times at 37° in the presence or absence of 1 mM theophylline. The reaction was stopped by placing the tubes in boiling water for 10 min. Following centrifugation, aliquots were applied along with unlabeled nucleotides to polyethylene imin-impregnated cellulose thin-layer plates. After the plates were developed with 0.1 M formate buffer, pH 3.3, spots observed with a UV lamp were removed from the plates, placed in PPO and POPOP scintillation fluid, and counted. Each determination was performed in duplicate; values represent the averages of two experiments.

RESULTS

Enhancement of Oxygen Uptake in Rat Heart Homogenates by Adenine Nucleotides—As shown in Table I, 0.17 mM cyclic 3′,5′-AMP, 5′-AMP, or 2′-AMP enhanced the rate of oxidation of octanoate, butyrate, or DL-β-hydroxybutyrate by rat heart homogenates approximately two or threefold. The rates of oxidation of the fatty acids were linear for 22.5 min. in the absence or presence of nucleotide. Data presented in Table I show that these nucleotides did not markedly alter the rate of oxidation of citrate, succinate, malate, or glutamate by heart homogenates. However, the rates of oxidation of pyruvate or α-ketoglutarate were enhanced by the nucleotides.

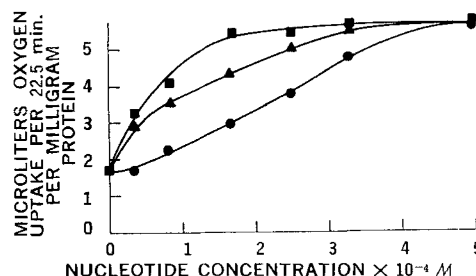

Figure 1—*Enhancement of octanoate oxidation by various concentrations of nucleotides. Conditions are as described in Table I and the text; each point represents the average of four experiments. Final concentrations of nucleotides are as shown. Key:* ■, *cyclic 3′,5′-AMP;* ▲, *2′-AMP; and* ●, *5′-AMP.*

Table IV—Enhancement of Carbon Dioxide Formation in Rat Heart Homogenates by Cyclic 3′,5′-AMP and 5′-AMP with 1-^{14}C-Octanoate as Substrate

Nucleotide	c.p.m.[a]	Stimulation, %
None	1105	—
Cyclic 3′,5′-AMP	3896	253
5′-AMP	3481	215

[a] For procedures, see text. Values are the average of three separate experiments.

Since it has been demonstrated that the presence of carnitine may influence the rate of oxidation of fatty acids (30, 31), the effects of this compound on the rate of fatty acid oxidation in the presence or absence of nucleotides were examined. Carnitine did not affect the rate of oxidation of butyrate or DL-β-hydroxybutyrate, but this compound produced a slight increase in the rate of oxygen uptake with octanoate as the substrate. The presence of carnitine did not alter the enhancement of oxygen uptake produced by the nucleotides with octanoate as the substrate. However, to assure that the concentration of carnitine would not be rate limiting in the oxidation of the fatty acids, all experiments were conducted with 0.1 m*M* carnitine in the reaction mixture. This concentration of carnitine was above optimal under these conditions.

It is known that heart contains the enzyme cyclic 3′,5′-phosphodiesterase, which catalyzes the conversion of cyclic 3′,5′-AMP to 5′-AMP (32). Therefore, all results presented in this paper were obtained with 1 m*M* theophylline, previously reported to be an inhibitor of cyclic 3′,5′-phosphodiesterase (33), in the reaction mixture. However, it has been reported that 1 m*M* theophylline is ineffective in completely inhibiting phosphodiesterase activity (33). Therefore, the possibility that the enhanced oxygen produced by 3′,5′-AMP may be due to conversion of this nucleotide to 5′-AMP was investigated. To examine this possibility, 8-^{14}C-cyclic 3′,5′-AMP[4] in a final concentration of 0.33 m*M* was incubated at 37° for various times with 0.5 ml. of 10% heart homogenate in a complete reaction mixture in the presence or absence of 1 m*M* theophylline. As shown in Table II, after 45 min. approximately 17.5% of the cyclic nucleotide was converted to 5′-AMP in the absence of theophylline. In the presence of 1 m*M* theophylline, 10% of the cyclic 3′,5′-AMP was converted to 5′-AMP. Inasmuch as only 10% of the added cyclic 3′,5′-AMP was hydrolyzed, the enhancement of oxygen uptake by the cyclic nucleotide probably was not due to 5′-AMP. In addition, if conversion of cyclic 3′,5′-AMP to 5′-AMP was involved in increasing octanoate oxidation, it is unlikely that linear rates of oxygen uptake would have been observed.

Enhancement of Oxygen Uptake in Rat Heart Homogenates by Various Concentrations of Nucleotides—As shown in Fig. 1 with heart homogenate, which was used 10 min. after preparation, the minimum concentration of cyclic 3′,5′-AMP or 2′-AMP that was effective in significantly augmenting octanoate oxidation by rat heart homogenates was 0.033 m*M*. A maximum enhancement was obtained with approximately 0.5 m*M* final concentration of the nucleotides. At low concentrations, 5′-AMP produced less enhancement of oxygen uptake than cyclic 3′,5′-AMP, but results obtained with 5′-ADP were virtually identical to those found with the cyclic nucleotide. When 5′-AMP and cyclic 3′,5′-AMP were added to the same reaction mixture, the percent stimulation of octanoate oxidation was additive at total nucleotide concentrations less than 0.5 m*M*.

The data presented in Table III show that the rate of oxidation of octanoate by heart homogenates in the absence of nucleotide was greatly diminished if the preparations were allowed to stand for 30 min. at 0° before use. Since the rate of oxygen uptake in the presence of nucleotide was not as markedly altered, the enhancement of octanoate oxidation produced by the nucleotides was increased from 3.3 to 24-fold for homogenates aged at 0° for 10 min. compared to those aged for 30 min.

To provide further indication of the ability of 5′-AMP or cyclic 3′,5′-AMP to enhance octanoate oxidation, the rate of $^{14}CO_2$-evolution from 1-^{14}C-octanoate was examined. As shown in Table IV, the amount of $^{14}CO_2$-evolution was increased 253% by 0.33 m*M* cyclic 3′,5′-AMP. When 5′-AMP was added to the incubation mixture, a 215% increase in $^{14}CO_2$-formation was observed.

[4] Schwartz Bio Research, Inc.

Table V—Effect of Various Nucleotides on Oxygen Uptake by Rat Heart Homogenates with Octanoate as Substrate

Nucleotide	Q_{O_2}[a]	Increase, %
None	1.90 ± 0.20	—
3′,5′-AMP	5.41 ± 0.36	185
5′-AMP	5.15 ± 0.27	171
5′-GDP	3.84 ± 0.29	102
5′-GTP	5.30 ± 0.39	179
5′-ADP	5.54 ± 0.41	192
3′,5′-Dibutyryl AMP	5.38 ± 0.37	183
2′-AMP	4.27 ± 0.16	125
2′,3′-AMP	1.90 ± 0.10	—
3′-AMP	2.60 ± 0.23	37
Adenosine	2.06 ± 0.12	—
Adenine	1.93 ± 0.20	—
3′,5′-GMP	2.70 ± 0.19	42

[a] Experimental conditions are as described in the text, and values are expressed as in Table I. Results are significant at the 95% probability level as shown by the Student *t* test.

Specificity of Action of Nucleotides—As shown in Table V, cyclic 3′,5′-dibutyryl AMP, 5′-ADP, or 5′-guanosine triphosphate (5′-GTP) was as effective as cyclic 3′,5′-AMP, 5′-AMP, or 2′-AMP in enhancing oxygen uptake by rat heart homogenates with octanoate as the substrate. However, cyclic 2′,3′-AMP, 3′-AMP, adenosine, or adenine produced little change in the rate of octanoate oxidation. In addition, cyclic 3′,5′-guanosine monophosphate (3′,5′-GMP) or 5′-guanosine diphosphate (5′-GDP) was not as effective in increasing the rate of oxygen uptake as the corresponding adenine nucleotides.

Effects of Nucleotides on Rat Heart Acyl Coenzyme A Synthetase—To determine the site of action of the nucleotides on fatty acid oxidation, studies were performed on the effects of cyclic 3′,5′-AMP or 5′-AMP on oxidation of octanoyl coenzyme A, butyryl coenzyme A, or acetoacetyl coenzyme A. As shown in Table VI, the rates of oxidation of these thioesters were not altered by the nucleotides. The results presented in Table VI are of particular interest when the rates of oxidation of the thioesters are compared with the rates of oxidation of the corresponding fatty acids. In the absence of the nucleotides, 5′-AMP or cyclic 3′,5′-AMP, octanoyl coenzyme A and butyryl coenzyme A were oxidized at rates appreciably faster than their corresponding fatty acids, indicating that under these conditions acyl coenzyme A synthetase activity is rate limiting.

Since it is likely that cyclic 3′,5′-AMP or 5′-AMP enhances the rate-limiting step in the oxidation of fatty acids, the effects of these nucleotides on a medium-chain fatty acyl coenzyme A synthetase from rat heart homogenates were investigated. As shown in Table VII, the activity of the enzyme with butyrate as the substrate was increased 100, 66, or 60% by 0.33 m*M* cyclic 3′,5′-AMP, 5′-AMP, or 2′-AMP, respectively. Nucleotide specificity similar to

Table VI—Effects of Nucleotides on the Rate of Oxidation of Acyl Coenzyme A Derivatives

Substrate[a]	Nucleotide	Q_{O_2}
Acetyl coenzyme A	None	0.90 ± 0.11
Acetyl coenzyme A	Cyclic 3′,5′-AMP	0.95 ± 0.13
Acetyl coenzyme A	5′-AMP	1.05 ± 0.09
Butyryl coenzyme A[a]	None	3.82 ± 0.19
Butyryl coenzyme A[a]	Cyclic 3′,5′-AMP	3.60 ± 0.37
Butyryl coenzyme A[a]	5′-AMP	3.80 ± 0.41
Octanoyl coenzyme A[a]	None	4.55 ± 0.26
Octanoyl coenzyme A[a]	Cyclic 3′,5′-AMP	4.61 ± 0.35
Octanoyl coenzyme A[a]	5′-AMP	4.32 ± 0.42
Acetoacetyl coenzyme A	None	2.00 ± 0.39
Acetoacetyl coenzyme A	Cyclic 3′,5′-AMP	2.12 ± 0.40
Acetoacetyl coenzyme A	5′-AMP	2.04 ± 0.39

[a] Of a number of concentrations used, 1.0 m*M* was found to give optimal rates of oxygen uptake. After 22.5 min., at least 70% of the added acylthioesters was present as measured by hydroxamate formation (25). Experimental procedures are as described in the text.

Table VII—Activation of Medium-Chain Fatty Acyl Coenzyme A Synthetase from Rat Heart Homogenate by Various Nucleotides

Substrate	Nucleotides	Thioester,[a] nanomoles
Butyrate	None	4.8
Butyrate	3′,5′-AMP	9.6
Butyrate	5′-AMP	8.0
Butyrate	2′-AMP	7.6
Butyrate	3′-AMP	5.2
Butyrate	2′,3′-AMP	4.0
Octanoate	None	10.8
Octanoate	3′,5′-AMP	20.0
Octanoate	5′-AMP	15.2
Octanoate	2′-AMP	14.8
Octanoate	3′-AMP	11.2
Octanoate	2′,3′-AMP	11.6

[a] Represents nanomoles of butyrohydroxamate or octanoylhydroxamate formed per 30 min. per milligram protein, based on a molar absorbance coefficient of 1000 for the acyl hydroxamates (29).

that obtained with oxygen uptake was observed in that 3′-AMP or cyclic 2′,3′-AMP was without effect on hydroxamate formation. It has been reported that the high concentrations of hydroxylamine used to determine the rate of thioester formation in crude tissues may inhibit the acyl coenzyme A synthetase reaction (29). The inhibitory effect of hydroxylamine might also prevent maximal activation of the enzyme by the nucleotides, which might account for the differences in enhancement of hydroxamate formation compared to the increase in oxygen uptake produced by the nucleotides.

Inasmuch as Bar-Tana and Rose (18, 19) observed that high concentrations of ATP activated a medium-chain fatty acyl coenzyme A synthetase from bovine liver and since it appeared that the activity of this enzyme was rate limiting in the overall rate of fatty acid oxidation by heart homogenates, the rate of oxygen uptake at various concentrations of ATP in the presence or absence of cyclic 3′,5′-AMP with octanoate as substrate was determined. As shown in Fig. 2, when the rate of oxygen uptake by heart homogenates is plotted as a function of ATP concentration, a sigmoidal curve is obtained with a maximum rate at 0.33 m*M* ATP. At this concentration of ATP, 0.33 m*M* cyclic 3′,5′-AMP was without effect; however, in the presence of the cyclic nucleotide the curve obtained was hyperbolic, showing that at concentrations of 0.02–0.1 m*M* ATP, cyclic 3′,5′-AMP markedly enhanced octanoate oxidation.

DISCUSSION

The initial step in the oxidation of fatty acids requires "activation" of the fatty acid by acyl coenzyme A synthetase to give an acyl coenzyme A thioester. This reaction is followed by β-oxidation of the acyl group, apparently requiring carnitine transferase and the β-oxidative enzymes (24). Since 5′-AMP, 5′-ADP, and cyclic 3′,5′-AMP enhanced the oxidation of fatty acids but were without

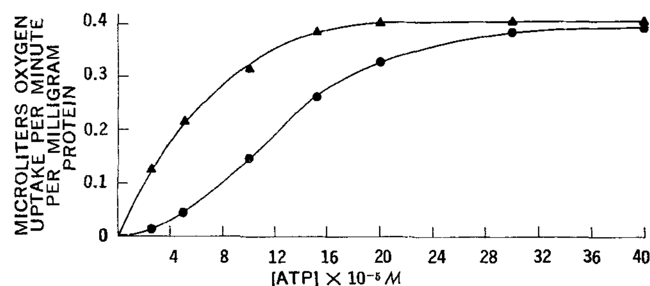

Figure 2—*Effects of cyclic 3′,5′-AMP on octanoate oxidation by rat heart homogenates as a function of ATP concentration. Conditions are as described in Table I. All rates were linear for 22.5 min., and the values of oxygen uptake per minute per milligram protein were calculated from the respective slopes. The final concentration of cyclic 3′,5′-AMP (▲) was 0.33* mM, *and each value represents the average of three experiments; ● represents values obtained in the absence of cyclic nucleotide.*

effect on the more rapidly oxidized thioesters, it is suggested that these nucleotides increase the rate of the acyl coenzyme A synthetase enzyme reaction. Direct evidence in support of this proposal was obtained by the observation that 5′-AMP or cyclic 3′,5′-AMP increased the rate of formation of acyl hydroxamates in rat heart homogenates.

As shown in the results, a sigmoidal curve was obtained when the rate of oxygen uptake was plotted as a function of ATP concentration in the absence of cyclic 3′,5′-AMP; in the presence of the cyclic nucleotide, the curve was hyperbolic. It would be interesting to speculate that these results may be due to a cooperative interaction of the nucleotides with acyl coenzyme A synthetase, particularly since Bar-Tana and Rose (18, 19) showed that ATP and 5′-AMP functioned as heterotropic cooperative ligands for the partially purified enzyme from bovine liver. This speculation is possible, because it appears that the rate of oxygen uptake is dependent upon the rate of the acyl coenzyme A synthetase reaction. However, inasmuch as crude heart homogenates were used in these studies, other explanations for the data are possible. Studies on the mechanism of activation of medium-chain acyl coenzyme A synthetase from heart by nucleotides are in progress, but the extreme lability of the enzyme makes such studies difficult. Also, reliable kinetic data are difficult to obtain due to the presence of acyl coenzyme A deacylase, even in the partially purified preparations, which interferes with the standard assay procedures.

Robison *et al.* (34) reported the normal levels of cyclic 3′,5′-AMP in rat heart to be approximately 0.4 μM; after perfusion with epinephrine, this level rapidly increased to 1.4 μM. The concentrations of 5′-AMP and 5′-ADP in heart tissues have been reported to be approximately 0.2 and 0.4 m*M*, respectively (35). The results reported here show that 0.2 m*M* 5′-AMP or 5′-ADP appreciably enhanced fatty acid oxidation and that higher concentrations (Fig. 1) were required for maximal activation. However, the concentration of cyclic 3′,5′-AMP in heart tissue is much lower than that required to augment octanoate oxidation in this study. To what extent these nucleotides are involved in the regulation of heart fatty acid oxidation remains to be elucidated.

SUMMARY

Cyclic 3′,5′-AMP, 5′-AMP, or 2′-AMP enhanced the rate of oxygen uptake by rat heart homogenates with octanoate, butyrate, or DL-β-hydroxybutyrate as the substrate approximately 3.0-, 3.1-, or 2.8-fold, respectively. In studies performed with 1-^{14}C-octanoate as the substrate, 5′-AMP or cyclic 3′,5′-AMP produced a 2.5-fold increase in the rate of $^{14}CO_2$-evolution by heart homogenates. However, these nucleotides did not alter the rate of oxidation of octanoyl coenzyme A, butyryl coenzyme A, or acetoacetyl coenzyme A. Similarly, these nucleotides produced little effect on the oxidation of various citric acid cycle intermediates or glutamate by rate heart homogenates. The rate of butyrate or octanoate oxidation by rat heart homogenates was not altered by cyclic 2′,3′-AMP, 3′-AMP, adenosine, or adenine. However, 5′-ADP, 3′,5′-dibutyryl-cyclic AMP, 5′-GDP, or 5′-GTP enhanced fatty acid oxidation. Under the conditions used, 5′-AMP, 2′-AMP or cyclic 3′,5′-AMP was effective in augmenting fatty acid oxidation at concentrations as low as 0.025 m*M*.

The nucleotides increased the activity of a medium-chain fatty acyl coenzyme A synthetase from rat heart homogenates, and it is suggested that the ability of the nucleotides to enhance fatty acid oxidation by heart homogenates is due to activation of acyl coenzyme A synthetase.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 26, 1970, from the *School of Pharmacy, University of Colorado, Boulder, CO 80302*
Accepted for publication July 27, 1970.
This work was supported by Research Grant MH-14519, National Institutes of Health, U. S. Public Health Service.

Vagal Adrenergic Degranulation of Enterochromaffin Cell System in Guinea Pig Duodenum

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Abstract □ Working under the hypothesis that sympathetic nerve stimulation is involved in the degranulation of enterochromaffin cells in the duodenum of the guinea pig, a series of experiments utilizing both chemical and immunological sympathectomy was performed. Once sympathectomy was established, histological examination of the duodenum revealed enterochromaffin cell granulation was unaffected by peripheral vagal stimulation. This would tend to suggest adrenergic, and not cholinergic, fibers are implicated in the degranulation process in the guinea pig. This does not conflict with previous reports that vagal stimulation induces degranulation, because it has been shown that sympathetic fibers run in the vagal trunk of the guinea pig.

Keyphrases □ Enterochromaffin cell system, guinea pig—vagal adrenergic degranulation □ Sympathectomy, chemical, immunological—guinea pig □ Serotonin release—vagal stimulation effect □ Vagal stimulation effect—enterochromaffin cells

It is well known that the duodenum is the predilection site of the enterochromaffin cell (EC) and serotonin (5-HT) occurrence in the alimentary canal of the guinea pig (1). On the other hand, the exact physiologic release mechanism of liberated 5-HT from the EC in the intestinal tract is still largely unknown. Previously, the authors reported (2) that adequate electrical stimulation of the cervical vagus peripherally produced a reduction in argentaffin-positive EC granularity in guinea pig duodena.

Although no synaptic contacts to EC have been detected in mouse colon glands, in cells containing granules characteristic of EC, continuity has been established in a few cases between their basal processes and mucosal nerve fasciculi (3). By fluorescent microscopy, Gabella and Costa (4) traced adrenergic fibers to epithelial structures in the guinea pig gut. Jacobowitz (5) also claimed to follow some fluorescent fibers from perivascular plexuses to the surface epithelium in the mucosa of the cat and monkey small intestine. Whether the EC system is directly influenced by these nerves or affected only by sympathetic transmitter released from adjacent perivascular nerves remains to be seen.

Data from a previous study (2) seemed to indicate that EC granulation may have occurred *via* a noncholinergic mechanism. This suggestion, plus the fact that sympathetic fibers within the vagus were reported for the cat (6), the dog (7), and others (8), supports the contention that adrenergic fibers present in the guinea pig vagus may have been stimulated. Therefore, the present study is designed to provide further information on the nature of vagal degranulation of the EC system in the guinea pig duodenum.

METHODS

Animals—Albino male guinea pigs of the same strain and about 400 g. were used.[1] After an appropriate acclimatization period and a 24-hr. fast from a commercial pellet food (Purina), each guinea pig

[1] Obtained from Marland Farms, Wayne, N. J.