Effects of Lipolytic and Antilipolytic Substances on Adenosine 3',5'-Monophosphate Levels in Isolated Fat Cells*

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SUMMARY

The actions of certain lipolytic and antilipolytic agents were studied in isolated fat cells at the level of cyclic AMP. The following observations were made.

Epinephrine (5.5 μM) produced small but significant changes in cyclic AMP levels in 10-min incubations while caffeine (1 mM) was without significant effect. In combination, epinephrine and caffeine acted synergistically, producing large increases in cyclic AMP, indicating that, as in other systems, the catecholamines act to stimulate adenyl cyclase and the methyl xanthines act to inhibit the cyclic nucleotide phosphodiesterase.

Other hormones which acted to increase cyclic AMP levels in the presence of 1 mM caffeine included adrenocorticotropic hormone (ACTH), glucagon, thyroid-stimulating hormone, luteinizing hormone, norepinephrine, and the synthetic catecholamine isoproterenol.

Increased levels of cyclic AMP were detectable within 30 sec after the addition of epinephrine to fat cells incubated with 1 mM caffeine and were maximal at about 6 min, after which they declined.

Supramaximal epinephrine and supramaximal ACTH together did not produce greater stimulation than either agent alone, which suggested that they might act at the same location.

Three compounds with antilipolytic activity were tested. Insulin decreased cyclic AMP levels in fat cells exposed to epinephrine, ACTH, or glucagon in the presence of caffeine, while the β-adrenergic blocking agent pronethalol was effective against epinephrine but not ACTH or glucagon under the experimental conditions used. Nicotinic acid decreased cyclic AMP levels in the presence of epinephrine.

Adenosine 3',5'-monophosphate has been implicated as an intracellular second messenger (1, 2) in the actions of epinephrine and other lipolytic agents and in the antilipolytic actions of insulin on the rat epididymal fat pad in vitro (3-5).

To summarize briefly the data which led to this conclusion, changes in the rate of lipolysis were related to changes in intracellular cyclic AMP levels in fat pads under a variety of experimental conditions. For example, incubation or perfusion of fat pads with lipolytic concentrations of epinephrine produced changes in cyclic AMP levels which were proportional to the concentration of the hormone and these changes in cyclic AMP occurred before increased lipolysis was detectable. Also, the action of epinephrine on both cyclic AMP and lipolysis was blocked by β-adrenergic blocking agents known to inhibit the adenyl cyclase system (3). The methyl xanthines, inhibitors of the cyclic nucleotide phosphodiesterase (6) which were first shown by Vaughan and Steinberg to act synergistically with epinephrine on lipolysis (7), also acted synergistically at the level of cyclic AMP. In addition, the derivative N6-2'-O-dibutyryl cyclic AMP (3, 8) as well as exogenous cyclic AMP mimicked the effects of epinephrine on lipolysis (3, 9, 10). Thus there appeared to be little doubt that the intracellular level of cyclic AMP determined at least in part the rate of lipolysis in fat cells.

However, adipose tissue is far from homogeneous. This was most apparent when the antilipolytic action of prostaglandin E1 was studied on the whole fat pad in vitro (5, 11). Although PGE1 antagonized the effects of lipolytic hormones on both cyclic AMP levels and free fatty acid release, as would be expected from data of Steinberg et al. (12), PGE1 alone caused increased intracellular levels of cyclic AMP (sufficient to activate lipolysis fully) and yet had no effect on free fatty acid release. This seemingly anomalous action of PGE1 has been clarified with the isolated fat cell preparation developed by Rodbell (13, 14). Although PGE1 antagonized the effects of lipolytic hormones on cyclic AMP levels in isolated fat cells, the stimulatory effect of the prostaglandin was lost after the fat cells were separated from the other cell types found in the fat pad (15).

Thus the importance of the use of a relatively homogeneous system when studying a fundamental substance like cyclic AMP, which is common to nearly all cells (1, 2, 16), became apparent. The isolated fat cell preparation offers other ad-
vantages in addition to homogeneity, for it is a system less liable to artifacts caused either by limited access of an agent to the cell or by imperfect tissue sampling

Therefore, some temporal, quantitative, and qualitative relationships among lipolytic and antilipolytic agents and cyclic AMP levels in the isolated fat cell have been examined and are described in this communication. The actions of prostaglandins on cyclic AMP levels in isolated fat cells and other tissues are described in an accompanying communication (15).

EXPERIMENTAL PROCEDURE

Preparation and Incubation of Isolated Fat Cells and Measurement of Cyclic AMP—Isolated fat cells were prepared essentially as described by Rodbell (13). Male Sprague-Dawley rats weighing between 150 and 200 g were anesthetized with pentobarbital and the epididymal fat pads were removed. The fat pads were cut into small pieces and incubated in polyethylene vials of 25-ml capacity in 3 ml of Krebs-Ringer bicarbonate buffer containing one-half of the usual amount of calcium, 3% drazylized albumin, and 10 mg of collagenase. The tissues were incubated at 37° with collagenase until the fat pads disintegrated (20 to 40 min). The entire contents of the vials were filtered through a fiber glass screen (mesh about 1.0 mm square). The material remaining on the screen was washed with approximately 5 ml of Krebs-Ringer bicarbonate buffer at 37° C and the total filtrate was centrifuged at 100 × g for 1 min at room temperature in a plastic tube. The precipitate and the supernatant fluid were removed by aspiration, leaving the isolated fat cells (which float at the top) in the tube. The cells were washed two to four times with the original volume of warm (37°) Krebs-Ringer bicarbonate buffer and centrifuged. Finally, the cells were resuspended in an appropriate volume of the buffer and transferred into 25-ml polyethylene vials with plastic pipettes for the experimental incubation. The vials were gassed with 95% O2-5% CO2 and theophylline was purchased from Schwartz Biochemicals, and purified as described earlier (3). The volumes of the additions (usually 50 µl) never exceeded 1% of the total volume of 0.05 N HCl containing purified tritiated cyclic AMP (3) (about 10,000 dpm) to each vial. The acid-fat cell mixture was immediately transferred to glass 30-ml tubes, mixed vigorously on a rotary mixer, and placed in a boiling water bath for 12 min. After cooling, the acid mixture was applied to a Dowex-50 column, 15 × 0.7 cm, which had been equilibrated with 0.05 N HCl. The starting material was allowed to run over the column, and then an amount of 0.05 N HCl sufficient to bring the volume of the collected run through to 25 ml was added to the reservoir. This first 25-ml fraction was discarded. Next, 40 ml of 0.05 N HCl were added to the reservoir and the total 40-ml eluate was collected as the cyclic AMP-containing fraction. Sufficient Tris buffer (pH 7.4) was added to this 40-ml fraction to bring the final concentration to 25 mm, and then the pH was adjusted to between 7.5 and 8.0 (phenol red indicator) with 1.0 N KOH. This neutralized fraction was then applied to a Dowex 2 column, 5.0 × 0.7 cm, which had been previously equilibrated with 0.01 M Tris buffer, pH 7.4. The total starting material was allowed to run through and the column was then washed with 15 ml of 0.01 M Tris, pH 7.4. The run through and wash fractions were discarded. The tips of the columns were carefully rinsed to clear away any contaminants and 50-ml serum bottles were placed under the columns. Exactly 20 ml of 0.05 N HCl were added to each column and the total eluate was collected in the serum bottles. The serum bottles were stopped tightly, and the contents were shell frozen and lyophilized to dryness. The contents of the serum bottles were redissolved in an appropriate amount of 0.01 M Tris (pH 7.4) (usually 1.0 ml) and the pH was adjusted to between 7.2 and 7.6. An aliquot (usually 10% of the volume in which the samples were redissolved) was sampled for counting so that the recovery of cyclic AMP could be estimated. The remainder of the final extract was then available for the estimation of cyclic AMP by the previously reported method involving the activation of inactive liver phosphorylase (3).

Dry weights of isolated fat cells were estimated by a method to be reported by Sneyd, Corrin, and Park (4). Aliquots (usually 0.5 ml) of the washed fat cell suspension were pipetted onto tared 25-mm Millipore filter papers (AAWP 02500, 0.8 µm, Millipore Filter Corporation, Bedford, Massachusetts), and the fluid phase was removed by vacuum filtration. The filter papers were dried over silica gel under reduced pressure for 12 to 24 hours and weighed. The averages of three determinations were used in calculating the weights of cells used in each experiment.

Preparation of Isolated Fat Cells—Collagenase was purchased from Worthington. Inulin was the 10 times recrystallized preparation prepared by the Novo Laboratorium (Copenhagen, Denmark). Powdered bovine Fraction V albumin was obtained from Nutritional Biochemicals, and prepared for use by dialyzing concentrated, neutralized solutions against glass-distilled water overnight at 4°. ACTH was the ACTHAR gel preparation from Armour Corporation (Chicago, Illinois) or ACTH, corticotropin injection (Parke Davis Company), and glucagon, Lot 258-234B/107/1/258, was a gift of Dr. Otto Behrens of the Eli Lilly Company. TSH (Lot NIH-TSH-B3), LH (Lot NIH-LH-134), and Pro- lactin (Lot NIH-PBI) were obtained through the courtesy of Dr. A. E. Wilhelm of the Endocrine Study Section, National Institutes of Health. Epinephrine-HCl, Piocin, and Pitressin were purchased in ampule form from Parke Davis. Caffeine and theophylline were purchased from Merck. Tritiated cyclic AMP was purchased from Schwartz Biochemicals, and purified as described earlier (3).

RESULTS

Effects of Epinephrine and Methyl Xanthines on Cyclic AMP Levels in Fat Cells—Epinephrine at a concentration of 5.5 µm caused a small but highly significant (p < 0.01) increase in cyclic AMP levels in isolated fat cells (Fig. 1), while 1 mm caffeine did not significantly change cyclic AMP levels. However, fat cells incubated in the presence of both epinephrine and caffeine contained very high levels of cyclic AMP. Thus in isolated fat cells, as in intact fat pads in vivo (3), the methyl xanthines and catecholamines acted synergistically on cyclic AMP accumulation. This synergistic effect of the methyl xanthines with lipolytic hormones is very probably related to the combination of the stimulation of adenyl cyclase by the hormone (16, 17) and the inhibition of the cyclase 3'-5' nucleotide phosphorylase by the methyl xanthine (4). Another pos-
sibility was that caffeine was facilitating the escape of cyclic AMP from the fat cells into the medium and hence away from phosphodiesterase activity, since tritiated cyclic AMP was not destroyed at an appreciable rate when added to the medium.

![Graph](image)

**Fig. 1.** The effects of epinephrine and caffeine on cyclic AMP levels in fat cells. Conditions were as described under "Experimental Procedure." The vertical lines in each bar are the standard errors of the mean.

![Graph](image)

**Fig. 2.** Effects of increasing concentrations of methyl xanthines on cyclic AMP accumulation. Conditions were as described under "Experimental Procedure."

![Graph](image)

**Fig. 3.** The time course of the effect of epinephrine on cyclic AMP levels in isolated fat cells incubated with 1 mM caffeine. The conditions were as described under "Experimental Procedure" except that the time of incubation after the addition of caffeine, control, or epinephrine solutions was as indicated. **O--O**, 1 mM caffeine plus 5.5 μM epinephrine; **●--●**, 1 mM caffeine. The vertical lines in each bar are the standard errors of the mean.

However, this did not appear to be the case, for in experiments similar to those of Fig. 1, in which the fat cells and media were separated and cyclic AMP was measured in both, most of the increased cyclic AMP in response to the combination of caffeine and epinephrine was found in the cells.

The synergistic effect of the methyl xanthines was concentra-

<table>
<thead>
<tr>
<th>Additions</th>
<th>Cyclic AMP (pmoles/g dry wt ± S.E.)</th>
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<tbody>
<tr>
<td>1 mM caffeine</td>
<td>410 ± 30</td>
</tr>
<tr>
<td>1 mM caffeine + ACTH, 200 milliunits/ml (8)</td>
<td>9,450 ± 670</td>
</tr>
<tr>
<td>1 mM caffeine + glucagon, 2 μg/ml (5)</td>
<td>4,080 ± 510</td>
</tr>
<tr>
<td>1 mM caffeine + thyroid-stimulating hormone, 20 μg/ml (3)</td>
<td>2,080 ± 170</td>
</tr>
<tr>
<td>1 mM caffeine + luteinizing hormone, 20 μg/ml (3)</td>
<td>6,900 ± 1590</td>
</tr>
<tr>
<td>1 mM caffeine + norepinephrine, 11 μM (3)</td>
<td>11,090 ± 1140</td>
</tr>
<tr>
<td>1 mM caffeine + isoproterenol, 11 μM (3)</td>
<td>11,550 ± 1150</td>
</tr>
</tbody>
</table>

*The numbers in parentheses are the numbers of experiments.*

### Table I

**Effects of 1 mM caffeine and variety of hormones on cyclic AMP levels in isolated fat cells**

Conditions are described under "Experimental Procedure."
Effect of Various Lipolytic Hormones on Cyclic AMP Levels in Isolated Fat Cells—Lipolytic hormones other than epinephrine were capable of stimulating the adenyl cyclase system in isolated fat cells (Table I). Among these were ACTH, glucagon, thyroid-stimulating hormone, luteinizing hormone, and, as would be expected, norepinephrine and the synthetic catecholamine isoproterenol. Agents which were tested but were without significant effect on cyclic AMP levels in isolated fat cells incubated with 1 mM caffeine included prolactin (5 μg per ml), Pitressin (1 unit per ml), and Pitocin (1 unit per ml).

Time Course of Effect of Epinephrine on Cyclic AMP Levels in Isolated Fat Cells—The response of isolated fat cells incubated with 1 mM caffeine and 5.5 μM epinephrine is very rapid (Fig. 3). The cyclic AMP level was increased by 30 sec, and continued to increase until 6 min, after which it fell slowly. The level of cyclic AMP was fairly well maintained between 10 and 20 min, and 10 minutes was adopted as the standard incubation time for reasons of convenience and reproducibility.

Effect of Increasing Epinephrine and ACTH Concentrations on Cyclic AMP Accumulation by Isolated Fat Cells—Cyclic AMP levels in isolated fat cells incubated with 1 mM caffeine and supramaximal levels of epinephrine, ACTH, or the combination of the two increased rapidly. The effect was more pronounced with epinephrine than with ACTH, and the combination of the two was even more effective. The effects of the methyl xanthines on cyclic AMP levels in fat cells incubated in the absence of epinephrine were small and somewhat variable. Even at the highest concentrations tested (10 mM) caffeine caused an increase of only 1.8-fold. However, it should be noted that this change in cyclic AMP levels, although numerically small, would be expected to cause a considerable activation of lipolysis, since the range of concentrations over which intracellular cyclic AMP is limiting in lipolysis is actually very narrow (3-5).
levels in fat cells incubated in the presence of 1 mM caffeine were significantly stimulated by epinephrine at a concentration of $5.5 \times 10^{-5}$ M (Fig. 4). At concentrations greater than $0.55 \mu M$, the isolated fat cells appeared to be fully stimulated and no further response was elicited. ACTH was effective at much lower concentrations under the experimental conditions used. The effects of the different concentrations of the hormone on cyclic AMP levels have been expressed as the percentage of maximal effect because the maximal levels of cyclic AMP were much higher in the experiments involving the ACTH than those involving epinephrine. This was probably due to the occasional variability of the response of different isolated fat cell preparations. Cyclic AMP levels in isolated fat cells from the same preparation incubated with either supramaximal epinephrine or ACTH were nearly identical.

**Effects of Supramaximal Levels of Lipolytic Hormones on Cyclic AMP Levels in Isolated Fat Cells**—The nonspecificity of the response of isolated fat cells to hormones suggested that there might be more than one adenyl cyclase system in adipose tissue (19). In an effort to test this hypothesis, isolated fat cells were incubated with supramaximal levels of epinephrine, ACTH, or the combination of the two hormones in the presence of 1 mM caffeine (Fig. 5). Isolated fat cells incubated with epinephrine or ACTH contained cyclic AMP levels approximately 30 times higher than the controls. However, cells incubated with both hormones did not exhibit any greater response. Similar results were obtained when supramaximal glucagon or thyroid-stimulating hormone were combined with supramaximal epinephrine.

**Effects of Insulin, Nicotinic Acid, and Pronethalol on Cyclic AMP Levels in Isolated Fat Cells**—Insulin depressed cyclic AMP levels in isolated fat cells exposed to epinephrine and caffeine (Fig. 6). This effect of insulin had been previously noted in intact fat pads (4) and in homogenates of epididymal fat pads which had been pre-exposed to insulin (20). As little as 0.01 milliunit per ml of insulin caused significant decreases in cyclic AMP levels in isolated fat cells. In addition, insulin antagonized the effects of glucagon (4 μg per ml) and ACTH (200 milliunits per ml) (Fig. 7).

Nicotinic acid is also an inhibitor of lipolysis (21). The

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### Table II

**Effect of nicotinic acid on cyclic AMP levels in isolated fat cells**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Cyclic AMP (pmoles/g dry wt, mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mM caffeine</td>
<td>210 ± 10</td>
</tr>
<tr>
<td>1.0 mM caffeine + 5.5 μM epinephrine</td>
<td>2270 ± 310</td>
</tr>
<tr>
<td>1.0 mM caffeine + 33 μM nicotinic acid</td>
<td>210 ± 10</td>
</tr>
<tr>
<td>1.0 mM caffeine + 5.5 μM epinephrine + 33 μM nicotinic acid</td>
<td>290 ± 30</td>
</tr>
</tbody>
</table>

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### Table III

**Effects of pronethalol on cyclic AMP levels in isolated fat cells**

The conditions used are described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Cyclic AMP (pmoles/g dry wt, mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>780 ± 30</td>
</tr>
<tr>
<td>0.5 mM pronethalol</td>
<td>1,650 ± 100</td>
</tr>
</tbody>
</table>
addition of 33 mM nicotinic acid to isolated fat cells resulted in an almost complete suppression ($p < 0.01$) of the increased cyclic AMP levels engendered by incubation with 1 mM caffeine and 5.5 mM epinephrine (Table II). Similar results were obtained in rat epididymal fat pads in vitro.

The $\beta$-adrenergic blocking agent pronethalol at 0.5 mM antagonized the actions of epinephrine on cyclic AMP accumulation in isolated fat cells just as in intact fat pads (Table III), as had been previously shown (5). However, the effects of ACTH and glucagon on fat cells were not antagonized by pronethalol.

**DISCUSSION**

Rodbell has made a contribution of great significance with the development and studies of the isolated fat cell system (13, 15, 22-24). While the relative homogeneity of the cell population is perhaps the greatest advantage of this preparation, the importance of unimpaired access of hormones to the cells and also of better sampling cannot be overestimated. The only disadvantage of the preparation noted in these studies was a quantitative variability. However, cells properly prepared always responded in the same fashion qualitatively.

The measurement of cyclic AMP at the low intracellular concentrations which appear to be physiologically relevant (3-5) would be of very great advantage even with the sensitive assays which are available (3, 25-27). By virtue of the synergistic effect that they exert in combination with lipolytic hormones, the methyl xanthines have provided amplification of the cyclic AMP response to hormones which stimulate adenyl cyclase, thus facilitating studies not only of these hormones but also of agents which act to decrease cyclic AMP levels. Measurements of lipolysis in these experiments were of little value for, in the presence of inhibitors, cyclic AMP levels were far in excess of those required for maximal lipolytic rates. However, the extrapolation of these data at high intracellular levels of cyclic AMP to lower levels, where cyclic AMP is rate-limiting on lipolysis, appeared to be justified. That this was correct was supported by experiments of Corbin, who showed a clear correlation between the effects of insulin on lowering cyclic AMP levels and inhibiting lipolysis in fat cells incubated with submaximal concentrations of epinephrine.

The factors which influence intracellular cyclic AMP levels in fat cells (and hence influence enzyme systems controlled in part by cyclic AMP, e.g., hormone-sensitive triglyceride lipase (28), phosphofructokinase (29), and phosphorylase (30, 31)) are so numerous that they are best presented in a schematic diagram (Fig. 8).

The lipolytic hormones (i.e., the catecholamines, ACTH, glucagon, thyroid-stimulating hormone, luteinizing hormone, and others) appear to act on the adenyl cyclase system rather than the phosphodiesterase. Although absolute proof of this awaits the preparation of a hormone-sensitive adenyl cyclase free of phosphodiesterase, several circumstantial lines of evidence support it. First, the synergistic effects of the hormones and methyl xanthines, which are known to inhibit the phosphodiesterase, strongly suggest that the hormones are stimulating adenyl cyclase. Second, the hormones stimulate the accumulation of cyclic AMP from exogenous ATP in cell-free preparations under conditions designed to minimize the participation of the phosphodiesterase. However, it must be recognized that, despite these data and the fact that these hormones have been without acute effects on the phosphodiesterase preparations available, this by no means rules out such effects. In most tissue the total concentration of phosphodiesterase (as measured in cell-free preparations) is much higher than adenyl cyclase (6, 18, 32, 33), and, in addition, the intracellular distribution of the phosphodiesterase is complex (6, 18). Therefore, it is not inconceivable that large changes in cyclic AMP levels might be affected with little, if any, changes in the phosphodiesterase activity measurable in homogenates or subcellular fractions. However, despite this possibility, the available evidence strongly supports the idea that the lipolytic hormones act on the adenyl cyclase system in fat, as they appear to do in tissues with which they are more commonly associated (32, 34-39).

If this is the case, then another dilemma presents itself. How can an adenyl cyclase system be stimulated by molecules of such disparate structures as epinephrine, ACTH, luteinizing hormone, and so on? Since the specificities of the adenyl cyclase systems of other tissues have been very exacting, it seemed possible that adipose tissue might contain two adenyl cyclases or more with different specificities. However, supramaximal concentrations of several hormones were as effective alone as in combination, suggesting that each was capable of fully activating the catalytic activity present in the cell. Thus these experiments did not support the multiple adenyl cyclase
system theory. However, they are subject to many qualifications. For example, it seems possible that the maximal accumulation of cyclic AMP in fat cells may be regulated by factors other than adenyl cyclase activity (e.g. substrate availability or phosphodiesterase activity). In addition, since adenyl cyclase does vary from tissue to tissue, it may be expected that some variation in a given tissue may be encountered in the future, just as several enzymes are known to differ within a single tissue.

β-Adrenergic blocking agents have been shown to antagonize the lipolytic response of adipose tissue to catecholamines and, at much higher concentrations, to polypeptide hormones as well (9, 40, 41). In our experiments, pronethalol caused a virtual obliteration of the effect of epinephrine on cyclic AMP but did not antagonize ACTH or glucagon. It seems possible that under the proper conditions inhibitory effects against the polypeptide hormones might have been shown. On the other hand, it is possible that the β-adrenergic blocking agents cannot antagonize actions of the polypeptide hormones on adenyl cyclase because of differences in affinity, for example, just as they are rather unsuccessful antagonists of glucagon on dog liver adenyl cyclase, although successful against catecholamines (36). In addition, Aulich, Stock, and Westermann (41) have shown that the high concentrations of either α- or β-adrenergic blocking agents required to antagonize the lipolytic actions of polypeptide hormones also antagonized the lipolytic actions of exogenous dibutyryl cyclic AMP and theophylline, which suggests that these drugs may be capable of inhibiting lipolysis at a point other than adenyl cyclase.

That insulin lowered cyclic AMP levels in isolated fat cells was not unexpected, since it has been shown to have this action on cyclic AMP in intact fat pads in vitro (40) and also by Jungas to decrease the accumulation of cyclic AMP by homogenates of fat pads which were exposed to insulin before homogenization (20). In addition, Jungas, who with Ball first described the antilipolytic effect of insulin (42), also showed that incubation of fat pads with insulin results in decreased active phosphorylase and increased concentrations of the I form of glycogen synthetase (20), both of which would be expected if intracellular cyclic AMP levels were decreased. Finally, a clear correlation between decreased cyclic AMP levels and the antilipolytic action of insulin has been recently shown by Corbin.

However, the mechanism by which insulin lowers cyclic AMP levels in the fat cells remains unclear. We, like Jungas (20), have been unable to elicit effects of insulin added to broken cell preparations after homogenization, and the turnover of cyclic AMP in fat cells is so rapid (15) that experiments with intact tissues have not yielded a clear answer. The data of Jungas suggest that insulin acts by inhibiting adenyl cyclase but do not eliminate stimulation of the phosphodiesterase. Conversely, Schultz, Senft, and Munske (43) have reported that insulin injection of alloxan diabetic rats resulted in rapid and significant restoration of liver and muscle phosphodiesterase activity as measured in homogenates. It also seems possible that insulin acts indirectly. For example, insulin might lower cyclic AMP through the formation of another intracellular second messenger or by altering the configuration of lipoproteins of the plasma membrane, as suggested by Rodbell (22), and this change then affects cyclic AMP levels.

The antilipolytic action of nicotinic acid on fat is well documented, and previously Krishna et al. (44) reported that nicotinic acid stimulated the phosphodiesterase in cell-free preparations. Our experiments have shown that nicotinic acid does lower cyclic AMP levels in adipose tissue, but do not identify the mechanism by which this occurs.

Finally, reports from several laboratories have suggested relationships among thyroid hormone, cyclic AMP, and lipolysis. Goodman and Bray have shown that fat pads from hyperthyroid rats are far more sensitive to lipolytic hormones than those from normals, and that fat from thyroidectomized rats is relatively refractory to hormonal stimulation (45). Further, Fisher and Ball (46) and Brodie et al. (47) have shown, with methyl xanthines in conjunction with lipolytic hormones, that the total amount of lipase is equal in the three thyroid states, but that the activation mechanism is defective. Brodie has reported that homogenates of adipose tissue from hyperthyroid rats contained more adenyl cyclase activity than normals (47), while Mandel and Kuehl reported that a very high concentration of triiodothyronine inhibited the phosphodiesterase in extracts (48). In any event, it does seem probable that intact fat tissue from hyperthyroid rats is more responsive (in terms of cyclic AMP) than hypothyroid.

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