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K. CYCLIC 3′,5′-AMP AND THE LIPOLYTIC EFFECTS OF HORMONES ON ADIPOSE TISSUE[1]

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After cyclic 3′,5′-AMP had been implicated in both the glycogenolytic response of the liver to epinephrine (E) and the steroidogenic effect of ACTH in the adrenal cortex, a study of the possible roles of the cyclic nucleotide in a variety of hormonal reactions was undertaken (17–19). One of the more attractive possibilities was the lipolytic response of adipose tissue. This effect, which has been the subject of a number of excellent reviews (14–16, 21, 23) was especially interesting for two reasons. First, it seems highly unlikely that increased free fatty acid (FFA) release could be directly related to phosphorylase activation. Thus, if the adenyl cyclase system were involved in the lipolytic response, it might provide an example of increased cyclic 3′,5′-AMP mediating a hormone action without involving phosphorylase. Second, the relative lack of specificity of adipose tissue for hormones was intriguing. In addition to the catecholamines (which were antagonized by both *alpha* and *beta* adrenergic blockers), several polypeptide and protein hormones were capable of stimulating FFA release. Several of these adipokinetic agents, ACTH, glucagon, vasopressin, and TSH, were known to cause increased accumulation of cyclic 3′,5′-AMP in tissues with which they were more commonly associated (17).

Some indirect evidence bearing on the lipolytic effect was available. Martha Vaughan had reported increased FFA release and phosphorylase activation in rat epididymal fat pads incubated with norepinephrine (NE), glucagon, and ACTH (20). Serotonin, on the other hand, produced phosphorylase activation but not increased lipolysis. Since fat tissue was known to possess an E-sensitive adenyl cyclase system (7, 18), these data might have been taken as a positive correlation between cyclic 3′,5′-AMP and lipolysis had it not been for the still unexplained effect of serotonin. Later Vaughan and Steinberg reported that caffeine, a known inhibitor of the cyclic 3′,5′-nucleotide phosphodiesterase (4), acted synergistically with E in stimulating FFA release (25). Coupled with the inhibition of the lipolytic effect by dichloroisoproterenol (DCI), which antagonized the E stimulation of adenyl cyclase from other tissues (9), the effect of the methyl xanthine provided strong suggestive evidence in favor of cyclic 3′,5′-AMP participating in the lipolytic effect. However, Vaughan and her co-workers felt that their data with regard to cyclic adenylate as a mediator of lipolytic hormones were at best equivocal (20-23, 25).

With these data as background we decided to test the hypothesis that cyclic 3′,5′-AMP was the intracellular second messenger in the lipolytic response of the

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rat epididymal fat pad. These experiments, which were done collaboratively between our group and Drs. R. J. Ho and H. C. Meng, began with an attempt to see if a qualitative, quantitative, and temporal correlation existed between cyclic 3′,5′-AMP and FFA release (2, 3).

Fat pads were paired and after a 20-min preliminary incubation in Krebs-Ringer bicarbonate buffer containing 3 % FFA-poor albumin, were transferred to fresh media and incubated with or without E. The incubation was stopped by dropping the fat pads in a small homogenizer containing 20 to 25 volumes (w/v) of 0.05 N HCl and tritiated cyclic 3′,5′-AMP, which was used to follow the recovery of the compound over three ion exchange columns used to separate cyclic 3′,5′-AMP from known and unknown inhibitors and activators of the biological assay of the compound. This assay, based on the ability of cyclic 3′,5′-AMP to increase the rate of conversion of inactive liver phosphorylase to the active form, has been recently modified so that as little as 1×10^{-6} μmole of cyclic 3′,5′-AMP can be conveniently detected (3). The incubation media were sampled for FFA determination by the method of Dole at the same time that the fat pads were removed.

Fat pads incubated in the absence of E for 20 min contained an average of 0.18 ± 0.008 μmoles of cyclic 3′,5′-AMP per g wet weight and FFA release was 12.6 ± 0.8 mμmole per g per min. The addition of 0.1 μg per ml E resulted in a 22% increase in cyclic AMP and an 80% increase in FFA release. When the E concentrations were 1.0 or 10 μg per ml the cyclic 3′,5′-AMP levels were 61% and 111% greater than the control, and FFA release 230% and 254%.

The addition of 1 mM caffeine to the incubation media produced a small change in cyclic 3′,5′-AMP levels and FFA release, but in fat pads incubated with caffeine and 0.1 μg per ml E, cyclic 3′,5′-AMP was increased by 139% and FFA by 257%. It was interesting that changes in cyclic 3′,5′-AMP of greater than 50% resulted in little additional FFA release. This suggested that once a certain level of cyclic 3′,5′-AMP was reached, one or more components of the system involved in FFA release was saturated, and thus cyclic 3′,5′-AMP was no longer rate-limiting.

The effects of adrenergic blocking agents on cyclic 3′,5′-AMP levels were also investigated. When 0.1 mM DCI was included in the incubation media, slight increases in the intracellular cyclic AMP levels were noted, and DCI at this concentration almost completely blocked the effect of 1.0 μg per ml E on cyclic 3′,5′-AMP accumulation and inhibited increased FFA release by 67%.

When fat pads were incubated with or without 10 μg per ml E for 5, 13, 20 and 40 min, intracellular cyclic 3′,5′-AMP was maximal at 5 min and remained at this level until 20 min, when it declined slowly. On the other hand, FFA release was not significantly increased until 13 min of incubation; however, our methods of detection may not have been sufficiently sensitive to detect an earlier change. Further evidence was obtained in fat pads perfused by the method of Ho and Meng (5), in which cyclic 3′,5′-AMP levels were increased 2.5-fold in pads perfused with 0.03 μg per ml E for 30 or 60 sec. These rapid increases in the intracellular levels of the cyclic nucleotide demonstrated that this change was fast

enough to precede or at least accompany lipase activation as measured by Vaughan *et al.* (24) as well as FFA release (2, 3).

While all these data were in agreement with the hypothesis that cyclic 3′,5′-AMP mediated the lipolytic response of the fat pad to E, a direct cause-effect relationship had not been demonstrated. Vaughan had shown earlier that exogenous cyclic 3′,5′-AMP was unable to mimic effects of E on the fat pad *in vitro* (20). This was not surprising, since cyclic 3′,5′-AMP was known to enter cells poorly and to be highly susceptible to inactivation by the cyclic nucleotide phosphodiesterase. Posternak has prepared several derivatives of cyclic 3′,5′-AMP, one of which is N^6-2′-0-dibutyryl cyclic 3′,5′-AMP (10). When fat pads were perfused or incubated with this compound, prompt and significant changes in FFA release and tissue FFA were noted, although cyclic 3′,5′-AMP itself was, as reported by Vaughan, without stimulatory effect on whole fat pads. Although cyclic 3′,5′-AMP stimulated lipolysis in isolated fat cells prepared by the method of Rodbell (12), the dibutyryl derivative stimulated it at least 10 times as much. Glycerol and FFA release by isolated fat cells in response to dibutyryl cyclic AMP were compared, and were found to approach 1:3 ratio at higher concentrations of the nucleotide. The effects of cyclic 3′,5′-AMP on isolated fat cells have been confirmed in a preliminary communication by Maickel *et al.* (8).

Rizack has reported that a lipolytic activity in cell free preparations from rat epididymal fat pad responded to cyclic 3′,5′-AMP in the presence of ATP and Mg^{+2} (11). Although the levels of cyclic adenylate required were very high compared to those in cells, and the effective concentration range of the cyclic compound was very narrow, evidence of such an effect in cell free preparations is of great interest.

Although it seems clear that the lipolytic effect of E is mediated by cyclic 3′,5′-AMP, the relationship between the cyclic nucleotide and the lipolytic effects of the other adipokinetic hormones has not been studied in much detail. In addition to the correlation between increased lipolysis and phosphorylase activation in the presence of ACTH, TSH and vasopressin [shown by Vaughan *et al.* (20, 22)], we have found that fat pads incubated in presence of ACTH had small increases in cyclic 3′,5′-AMP levels as compared to controls (2). Hynie *et al.* (6) reported that theophylline enhanced the lipolysis induced by NE, ACTH, and other adipokinetic substances. Thus these data seem to indicate that the polypeptide hormones also act *via* cyclic 3′,5′-AMP. However, the most convincing proof would be the responsiveness to these hormones as well as the catecholamines of washed, cell-free preparations of the adenyl cyclase system.

The influence of thyroid hormone on lipolysis has been receiving some attention lately. Using the methyl xanthine theophylline to obtain maximal lipase activation, Hynie *et al.* reported that the total lipolytic activity of adipose tissue from hyper- or hypothyroid rats was unchanged, and that the increased or decreased lipolytic response of these tissues was due to modified responsiveness to NE. They suggested that this changed responsiveness might be due to changes in adenyl cyclase levels (6). Data recently obtained in collaboration with Dr. G. A. Bray support the idea that thyroid hormone exerts an effect on the intra-

cellular level of cyclic 3′,5′-AMP. The expected increase in cyclic 3′,5′-AMP in fat cells from thyroidectomized rats was less than one third as much as those from normal rats in 5-min incubations with 1.0 μg per ml E (1). It is difficult at this time, however, to say which of the enzyme systems controlling cyclic adenylate levels might be modulated by the thyroid function. Experiments to determine if these systems are modified in hypothyroid animals are now in progress.

Figure 1 is a synthesis of the data currently available, and represents a summary of the known or probable interrelationships between adipokinetic hormones, cyclic 3′,5′-AMP, and lipolysis in adipose tissue. First, it seems quite clear that the catecholamines stimulate the adenyl cyclase system and thus increase intracellular cyclic 3′,5′-AMP. DCI and some other adrenolytic agents antagonize the effect of E on adenyl cyclase, and the methyl xanthines enhance the accumulation of the cyclic nucleotide by inhibiting the phosphodiesterase which destroys it.

One of the consequences of augmented intracellular cyclic 3′,5′-AMP is increased lipolysis, which is apparently effected *via* lipase activation. Although phosphorylase activation is another consequence, there is little reason to believe that this enzyme is directly related to the breakdown of triglycerides. Rather, it would seem that this control of lipolysis is another of the many effects of the cyclic nucleotide, and, like the inotropic effect (13, 19), is not dependent on phosphorylase activation.

It is probable that several other adipokinetic hormones act upon adipose tissue adenyl cyclase. Since the enzyme has shown a high degree of specificity in all other tissues which have been investigated to date (17–19), it will be interesting to see if fat has a single adenyl cyclase with a structure capable of transducing information from such dissimilar molecules or if there are several versions of the enzyme in this tissue. Finally, thyroid hormone appears to be capable of modifying the activities or levels of one or more of the enzymes which determine the concentration of cyclic 3′,5′-AMP in the fat cell. Thus it is manifest that the level of cyclic 3′,5′-AMP in adipose tissue is a crossroads between a variety of hormones and should be an extremely helpful model in the study of hormone actions and interrelationships at the molecular level.

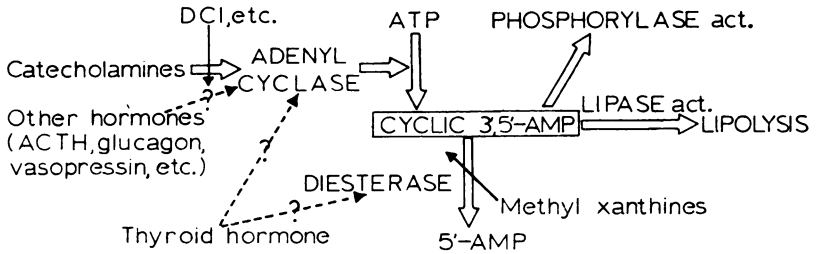

FIG. 1. Cyclic 3′,5′-AMP and lipolysis

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