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RAPID AND REVERSIBLE ACTIVATION OF ACETYL COA HYDROLASE IN INTACT PINEAL CELLS BY DISULFIDE EXCHANGE

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ABSTRACT: Using intact pinealocytes in suspended cell culture it has been determined that acetyl CoA hydrolase activity can be rapidly increased by treatment with cystamine. Similar results are seen with diacetylcystamine, but not with GSSG, penicillamine disulfide, nor with oxidized DTT. The activation of acetyl CoA hydrolase by cystamine is reversible: after cystamine treatment is terminated, enzyme activity decreases slowly in cell culture. It is also possible to reverse the activation by treating homogenates of cystamine-treated cells with dithiothreitol. These observations are consistent with previous findings indicating that pineal acetyl CoA hydrolase activity can be regulated *via* protein thiol:disulfide exchange. The observations presented in this report also indicate that conditions within the cell allow this type of reaction to take place, and raise the possibility that disulfide exchange mechanisms may be physiologically involved in the intracellular regulation of the activity of this and perhaps other enzymes.

Introduction

Interest in protein thiol:disulfide exchange as a mechanism of enzyme regulation has received renewed attention recently (1). It has been found that some enzymes are activated and others inactivated by this reaction. For example, disulfide exchange leads to the activation of fructose 1,6-disphosphatase, and the inactivation of glycogen synthetase, phosphorylase phosphatase and adenylate cyclase (2-5). The idea that disulfide exchange does play a role in the physiological regulation of enzyme activity was based solely on results of experiments using broken cell preparations; there is little direct evidence that this type of covalent modification of enzymes can take place in intact cells.

In recent studies using broken cell preparations, we have found that the activities of two enzymes in the pineal gland, serotonin N-acetyltransferase and acetyl CoA hydrolase, are altered by disulfide exchange (6,7). N-Acetyltransferase activity is rapidly decreased by treatment with cystamine

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or the disulfide peptide arginine vasotocin, whereas acetyl CoA hydrolase activity is rapidly increased by treatments with these compounds. In addition, we found that cystamine can inactivate N-acetyltransferase in intact pinealocytes, a finding which represents the first direct demonstration that the activity of an enzyme can be altered by this mechanism in an intact cell (6).

In the present report we have investigated the possibility that the activity of acetyl CoA hydrolase is also increased in intact cells treated with cystamine. The control of the activity of this enzyme, which converts acetyl CoA to CoA and acetate is of special interest in the pineal gland because of the dual role acetyl CoA has in the production of the putative pineal hormone, melatonin. Acetyl CoA is the acetyl donor, participating in the N-acetylation of serotonin by serotonin N-acetyltransferase and also stabilizes this unusually unstable enzyme. Serotonin N-acetyltransferase is also important because it controls large and rapid changes in the rate of production of melatonin.

The results of the study presented here clearly indicate that the activity of pineal acetyl CoA hydrolase in intact cells can be rapidly increased by treatment with cystamine, and that cystamine acts through a mechanism of disulfide exchange.

MATERIALS AND METHODS

Materials: The commercial sources of chemicals used in this study have been given (6), and the synthesis of N,N'diacetylcystamine has been detailed (7).

Methods: Pinealocytes were prepared from pineal glands taken from 2-day-old rats (8), and incubated for 24 h prior to treatment. The details of experimental manipulations are given in the legends to the figures.

Acetyl CoA hydrolase was measured by a radiochemical technique (7). Protein was determined by a dye-binding procedure, using serum albumin as a standard (9).

RESULTS

After 24 h of cell culture, the activity of acetyl CoA hydrolase in pinealocytes was about 2.0-3.0 nmol/min/mg protein. Treatment with cystamine (10 mM) resulted in activation of the enzyme (Table 1). The acti-

Table 1

Reversal of the cystamine activation of acetyl-CoA hydrolase by DTT treatment

Experimental treatment: Cells	Enzyme activity nmol/min/mg protein Homogenate -DTT	+DTT
None	3.0	4.4
Cystamine, 10 mM, 30 min	8.1	3.1

Pinealocytes were prepared as described (8). After 24 h of culture period the cells were treated with cystamine for 30 min. The cells were then centrifuged (0.5 min, 12,000 g), the supernatant removed, 10 µl of 0.1 M sodium phosphate buffer was added and the tubes were placed on dry ice. The cells were sonicated in 20 µl of the buffer and two sets of tubes were pooled, centrifuged and the supernatant removed. A 20 µl aliquot was incubated (37°C, 10 min) with 5 µl of DTT (50 mM) and the other 20 µl aliquot was incubated with the buffer. The enzyme activity was then determined in a total volume of 50 µl ([AcCoA]=5 mM). The values given are the mean of three samples of cells incubated and assayed separately.

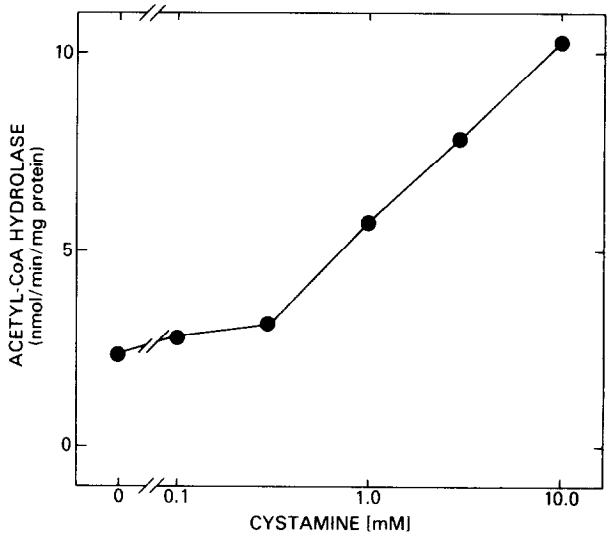

Figure 1. Dose-dependent increase in acetyl-CoA hydrolase in pinealocytes treated with cystamine. Treatment with cystamine was done as described in the legend to Table 1. The activity of acetyl-CoA hydrolase was measured as described (7). The values given are the mean of three individual samples of cells. The values were within 5% of the mean.

vated preparation of cells was sonicated and treated with DTT (10 mM). This reversed the activation produced by cystamine, and indicated that cystamine probably activated the enzyme by a mechanism of disulfide exchange (Table 1).

The effect of cystamine was further characterized. First, a dose-response analysis indicated that the activation of acetyl CoA hydrolase by cystamine was concentration-dependent (Figure 1). A time course study indicated that the effect of cystamine was extremely rapid, with marked activation apparent within 2 min after the addition of the compound (Figure 2). As expected, the activation occurs more rapidly in the homogenates than in intact cells (Figure 2). The specificity of cystamine

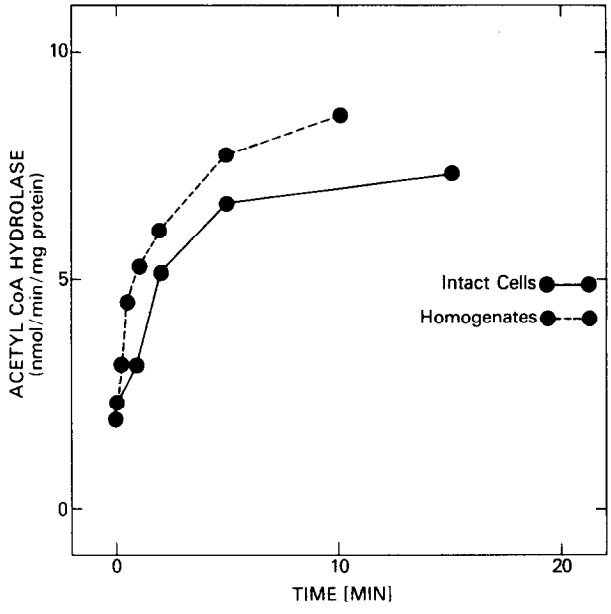

Figure 2. Time-course of activation of acetyl-CoA hydrolase by cystamine in pinealocytes and in broken cell preparations. The experiment using pinealocytes was done as described in Table 1 with the following modifications. The cells were treated with cystamine (10 mM) for the times indicated. They were pelleted and washed once with control medium (500 µl) and then frozen on dry ice. The enzyme assay was done in a total volume of 100 µl ([AcCoA] = 5 mM). In the broken cell experiment, pineal glands from adult rats were used. The glands were homogenized in 0.1 M sodium phosphate buffer pH 6.8 (1 gland/10 µl) and the assays were done as described (7). The activation was done in a 10 µl volume ([cystamine] = 1 mM, pH 8.5) and the assay was performed in 100 µl volume ([AcCoA] = 5 mM). The values given are the mean of three individual samples of cells (for the cell experiment) and the mean of duplicate determinations of samples of homogenates, which were within 5% of the mean.

Table 2

Activation of pineal acetyl CoA hydrolase in cell culture by cystamine and related compounds.

Test compound [10 mM]	Enzyme activity nmol/min/mg protein
None	2.9
cystamine	9.0
diacetyl cystamine	7.8
penicillamine disulfide	3.4
Oxidized DTT	2.8
GS-SG	3.7
G-SH	3.0

Cells were treated with the indicated compounds as described in the legend to Table 1. The values given are the mean of three samples of cells incubated and assayed separately.

was analyzed by treating cells with a number of disulfides. Cystamine was clearly the most potent compound (Table 2). CoA disulfide, which is inactive in broken cell preparations as regards this enzyme (7), was not used in these studies.

It was of interest to determine if the effects of cystamine were reversible in the intact cell. To examine this, cells were treated with cystamine for 20 min, centrifuged, and resuspended in medium without cystamine. This resulted in a rapid partial reversal, followed by a more gradual decrease in enzyme activity (Figure 3A). This suggested that the modification of the enzyme effected by cystamine was reversible in the intact cell. However, it is possible that the decrease in activity observed on removal of cystamine was due to the degradation of the cystamine-modified acetyl CoA hydrolase proteins and not due to the reversal of cystamine activation. To determine if degradation of acetyl CoA hydrolase protein is sufficiently rapid to cause a decrease in the activity of the enzyme, we measured the

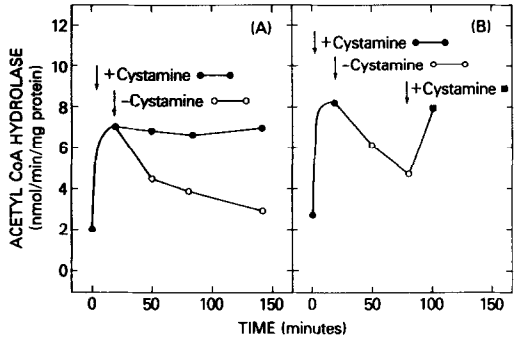

Figure 3. Reversal of cystamine-activation of acetyl-CoA hydrolase in intact pinealocytes. The values given are the mean of three samples of cells incubated and assayed separately. The values were within 5% of the mean. (A) Pinealocytes were treated with cystamine as described in Table 1. After 20 min the cells were pelleted, the supernatant removed and the cells were resuspended in cystmaine-free medium to test for reversal. The cells were then incubated as indicated. Another set of tubes were treated identically but were resuspended in cystamine-containing medium. (B) Pinealocytes were treated as in (A), except that following a 60 minute reversal period cells were again treated with cystamine for 20 minutes.

decrease in activity after protein synthesis was blocked by cycloheximide treatment (data not presented). No significant decrease in enzyme activity was found in either control or in cystamine-treated preparations up to 3 h, a period in which the reversal of cystamine activation was almost complete. This indicates that degradation of the enzyme is not rapid enough to explain the decrease in enzyme activity seen after cystamine was removed. Therefore, it appears probable that reduction of the mixed disulfide form of the enzyme inside the cell causes the observed decrease in activity.

It should be added that we have found it is possible to restimulate acetyl CoA hydrolase following a cycle of cystamine-activation and reversal (Figure 3B). The activation was essentially the same in both cases (7.8 vs 8.2 nmol/min/mg protein). This is consistent with the conclusion that during reversal the activated enzyme is converted to a less activated form, not that the activated enzyme is destroyed. Based on these observations, it would appear that acetyl CoA hydrolase might exist in two forms in equilibrium, the balance determined by mechanisms involving disulfide exchange.

It has been shown that dibutyryl cAMP increases mixed disulfide formation of proteins with GSH in the liver (10). Therefore, it was of interest to find out if dibutyryl cAMP has any effect on the activation of acetyl CoA hydrolase by cystamine or on its reversal. Addition of dibutyryl cAMP (1 mM) did not change the dose response of activation of the enzyme by cystamine (data not given). Also dibutyryl cAMP (1 mM) or norepinephrine (1 mM) treatment did not have a significant effect on the reversal of cystamine activation in the pinealocytes (data not given).

DISCUSSION

The results presented in this report clearly show that cystamine can activate acetyl CoA hydrolase by disulfide exchange inside pineal cells. An impressive feature is the extremely rapid nature of this activation. The rapid nature of disulfide exchange makes it an excellent mechanism for the rapid regulation of enzyme activity. However, the requirement for unphysiological concentrations of cystamine makes one question whether this type of reaction is of any physiological significance. We believe it is valuable to entertain this idea. If cystamine is regarded only as a model compound, one can imagine that more potent and specific disulfide peptides or proteins could be involved in regulatory reactions. It is known that mixed disulfides of proteins and glutathione or cysteine are present in quite high intracellular concentrations (11). Thus, although we feel that it is unlikely that cystamine is involved in the physiological control of enzyme activity *via* disulfide exchange, it seems likely to us that this mechanism does play a role in the intracellular regulation of metabolic pathways. It should be added that the ratio of protein-S-SG to GSH appears to be controlled by cyclic AMP in the rat liver, and that when cyclic AMP levels increase, the ratios of protein-S-SG to GSH also increase (10).

If one entertains the possibility that the activity of enzymes is controlled by disulfide exchange reactions, the next question that arises is whether the reaction is purely chemical or if it is catalyzed by an enzyme.

Several enzymes catalyzing this type of reaction have been described (1,12). However, the lack of good methods of assaying them has prevented substantial progress in this area.

As a final note, the broad potential of disulfide exchange in enzyme regulation needs to be mentioned. In actively metabolizing tissues the ratio of GSH to GSSG is greater than 20 (13). Presumably, the concentration of the disulfide, GSSG can be significantly changed by a shift in the redox state of the cell (GSH→GSSG). Since the exchange reactions are rapid, even a transient shift in redox state of the cell might bring about changes in the activity of many enzymes *via* this mechanism. Disulfide exchange reactions can also be controlled by other factors, including the activity of the enzymes catalyzing these reactions and the pH of the cytosol. These possibilities deserve further investigation, especially in neural tissue where rapid changes in enzyme activity are likely to be important components of normal function.

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