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ACTIVATION OF PINEAL AND BRAIN ACETYL-COA HYDROLASE BY CYSTAMINE: AN APPARENT CASE OF DISULFIDE EXCHANGE

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ABSTRACT: Rat pineal acetyl-CoA hydrolase was activated about 5-fold by cystamine treatment (30 mM) at pH 6.8 and 10-fold at pH 8.5. Six other disulfides were found to be ineffective or to produce a small activation. Cystamine activation was not reversed when free cystamine was removed, but was reversed by treatment with DTT. Analysis of other tissues indicated acetyl-CoA hydrolase from rat brain, sheep pineal gland and chicken pineal gland could also be activated by cystamine. In contrast, cystamine activation of rat liver acetyl-CoA hydrolase was not seen.

Introduction

Acetyl-CoA hydrolase (EC 3.1.2.1) may be an important factor controlling the concentration of acetyl-CoA in cells (1). In the pineal gland, acetyl-CoA appears to have two key roles in melatonin production (2). First, it serves as the acetyl donor in the conversion by serotonin N-acetyltransferase (EC 2.3.1.5) of serotonin to N-acetylserotonin (3), the precursor of melatonin. Secondly, acetyl-CoA is the most potent known stablizer of pineal serotonin N-acetyltransferase, an unusally labile enzyme (4,5). This enzyme is of interest because the activity of it increases 30- to 70-fold at night in the rat and chicken (6,7), resulting in large changes in N-acetylserotonin and melatonin production (2).

We had sought in a previous report to determine if the activity of acetyl-CoA hydrolase changes under a number of conditions in which the activity of N-acetyltransferase exhibits large changes (8). Marked changes in acetyl-CoA hydrolase activity, however, were not found. This led to our speculation that acetyl-CoA hydrolase activity in the cell might change via a reversible activation mechanism, not readily detectable in broken cell preparations (8). In the

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course of investigating the regulation of acetyl-CoA hydrolase activity, we found that cystamine activates this enzyme; these results are presented in this report. This finding leads to the hypothesis that acetyl-CoA hydrolase can be activated through disulfide exchange, a covalent modification which is receiving renewed interest as a mechanism of enzyme regulation (9).

Materials and Methods

Materials: Acetyl-CoA was purchased from Schwartz/Mann, New York, New York, and 1-[^{14}C]acetyl-CoA from New England Nuclear Corporation, Boston, Massachusetts. 1-[^{14}C]Acetyl-CoA was routinely lyophilized prior to use to remove contaminating 1-[^{14}C]acetic acid. Decolorizing charcoal (Norit A) was obtained from Fisher Scientific Co., New York, New York. N,N'-Diacetylcystamine was synthesized; the identity of the product was confirmed by mass spectroscopy. All other reagents used were of analytical grade.

Male Sprague-Dawley rats (125 to 150 g, Zivic-Miller, Allison Park, PA.) used for the experiments were maintained for 3 to 7 days prior to killing in L:D 14:10 lighting cycle; the light period started at 0500 hours. The animals were given food and water ad lib.

Methods: Rats were killed by decapitation at 1100 hours. Tissues were immediately removed and placed in a storage container on dry ice. Pineal homogenates were prepared by sonicating one or two glands in 20 µl of either 0.1 M sodium phosphate buffer, pH 6.8 or in 0.01 M veronal-HCl buffer, pH 8.5. Homogenates were centrifuged (0.5 min, 12,000 g) prior to analysis, except in the experiment presented in Table 2. More than 90% of the total activity of the enzyme was recovered in the supernatant fraction. The preparation of brain, liver and pineal glands from sheep and chicken is described in the legend to Table 2.

To measure enzyme activity, 10 µl of the supernatant was added to a tube containing 10 µl of 0.1 M sodium phosphate buffer, pH 6.8, and 100 nmol of 1-[^{14}C]acetyl-CoA (specific activity = 0.4 Ci/mol). The tube was incubated (20 min, 37°) and processed as described (10), except that a 100 mg/ml suspension of charcoal in 0.01 M sodium acetate was used to adsorb 1-[^{14}C]acetyl CoA.

Enzyme activity is presented on a per mg protein basis. Protein was measured by the dye binding method (11).

Results

Treatment with cystamine caused a dose-dependent increase in enzyme activity (Figure 1). This activation was greater at pH 8.5, as compared to pH 6.8. The maximum effect of cystamine (30 mM) was a 10-fold increase in activity.

The identity of the product formed by the unactivated enzyme in the assay used has been demonstrated to be 1-[^{14}C]acetate (10). In the present investigation we verified that the product formed by the cystamine-activated enzyme was 1-[^{14}C]-acetate, as indicated by thin-layer chromatographic analysis (ethanol: 1 M ammonium acetate, 5:2; R_f= 0.62 on precoated silica gel plates, F-254, Brinkman Instruments) and the complete volatilization of the product upon evaporation.

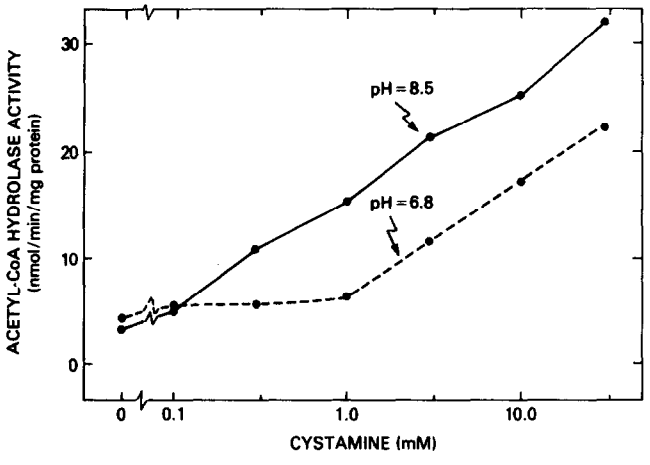

Figure 1. Dose-dependent activation of acetyl-CoA hydrolase by cystamine treatment. A sample of pineal homogenate equivalent to half a gland was incubated (30 min, 37°C) with the indicated concentration of cystamine in a total volume of 10 µl (0.1 M sodium phosphate, pH 6.8 or 0.01 M veronal-HCl buffer, pH 8.5). Enzyme activity was then assayed as described in the Methods Section. Analyses were run in duplicate, the results of which were within 5% of the mean.

The effect of several compounds related to cystamine was determined (Table 1). In contrast to the marked activating effect of cystamine, we found N,N'-diacetylcystamine, GS-SG, penicillamine disulfide, cystine, oxidized DTT and CoAS-SCoA were not capable of causing a large activation. In addition, the following thiols were either inactive or slightly inhibitory: cysteamine, penicillamine, cysteine, DTT and CoA. Treatment of the enzyme with diamines, including putrescine, cadaverine or diaminohexane, also did not increase activity. We did observe that in contrast to other thiols, GSH treatment activated the enzyme. This was verified by thin-layer chromatographic analysis of the product.

A limited tissue and species survey for the presence of cystamine-activatable acetyl-CoA hydrolase was performed (Table 2). Cystamine activated acetyl-CoA hydrolase in crude homogenates of rat brain and pineal glands from sheep and chickens. In contrast, cystamine slightly reduced rat liver acetyl-CoA hydrolase activity. In all cases the identity of the enzymatic product as [^{14}C]acetate was verified by thin-layer chromatography.

Table 1. Comparative study of the activation of acetyl-CoA hydrolase by cystamine and related compounds.

Test compound[1]	Acetyl-CoA hydrolase (nmol/min/mg protein) pH 6.8[2]	pH 8.5[2]
None	5.5	4.4
Disulfides		
cystamine	19.5	11.8
N,N'-diacetylcystamine	9.8	5.7
GS-SG	5.9	4.6
penicillamine disulfide	5.4	4.1
cystine		5.6
trans-4,5-dihydroxy-1,2-dithiane[3]	4.5	
CoAS-SCoA	4.2	
Thiols		
cysteamine	2.6	5.1
G-SH	10.2	4.5
penicillamine	4.1	3.3
cysteine	4.5	4.0
DTT	4.1	2.9
CoA	2.7	2.8
Diamines		
putrescine	5.4	5.0
cadaverine	5.7	5.0
1,6-diaminohexane	4.8	5.1

Samples of pineal homogenate were treated with the indicated compounds as described in Fig. 1. Enzyme activity was determined as described in the Methods Section. Experiments were run in duplicates, the results of which were within 5% of the mean. These results were obtained from two experiments, the first performed using 0.1 M sodium phosphate buffer, pH 6.8, and the second using 0.01 M veronal-HCl, pH 8.5.

[1]The concentrations of the test compounds were 10 mM in the pH 6.8 study and 1 mM in the pH 8.5 study.

[2]The pH of the solutions during activation only and not during assay.

[3]Oxidized DTT.

Cystamine activation of rat pineal acetyl-CoA hydrolase was reversed by reduction with DTT (Table 3). It seemed possible that DTT was either converting cystamine to the inactive cysteamine, or that DTT was reducing a disulfide formed between cystamine and the enzyme. The latter appeared to be a more likely explanation because of two observations. First, when free cystamine was completely removed by gel filtration, activation was not completely

Table 2. The effect of cystamine on acetyl-CoA hydrolase activity in different tissues.

Animal	Tissue	Acetyl-CoA hydrolase (nmol/min/mg protein)	
		Control	Cystamine[1]
Rat	Pineal gland	3.0	20.6
	Brain	3.6	6.8
	Liver	9.7	7.2
Sheep	Pineal gland	3.2	7.4
Chicken	Pineal gland	2.7	25.0

Activation and assay were done as described in Fig. 1. Sheep pineal glands were taken from adult male sheep (180 lbs.) killed in the dark using a high dose of Somlethol (Med. Tech. Inc., Elwood, KS.). Chicken pineal glands were taken from adult chickens killed by decapitation during the day. Homogenates were prepared in 0.1 M sodium phosphate buffer, pH 6.8, and were used without centrifugation.

[1]The concentration of cystamine was 30 mM.

reversed. There was, however, a 30 to 50% loss of activity of both the control and activated preparations as a result of gel filtration. It seems reasonable that this may be primarily due to non-specific effects because the ratio of enzyme activity in the activated preparation to that in the control preparation was not altered by gel filtration. In addition, it was determined that after free cystamine was removed from an activated preparation by gel filtration it was still possible to reverse the activation by DTT treatment (Table 3).

Discussion

The results of these studies clearly demonstrate that rat pineal acetyl-CoA hydrolase activity can be increased by cystamine treatment. In addition, this effect of cystamine, being observed in preparations of pineal glands from two other species, is not unique to the rat gland. Neither is it unique to the pineal gland, as activation was easily detectable in rat brain. Perhaps cystamine-activation of acetyl-CoA hydrolase activity may be common to neural tissue in general.

The results of our investigations lead to the hypothesis that cystamine activates pineal and brain acetyl-CoA hydrolase by protein thiol-disulfide exchange:

$$E\text{-}SH + RS\text{-}SR \rightleftharpoons ES\text{-}SR + RSH.$$

Table 3. Reversibility of cystamine activation of acetyl-CoA hydrolase by reduction (DTT treatment)

Experiment	Treatment	Acetyl-CoA hydrolase (nmol/min/mg protein)	
		Control	Activated[1]
I	None	3.0	13.4
	Reduction[2]	3.6	3.8
II	None	3.0	20.7
	Gel filtration[3]	2.1	12.9
	Gel filtration[3] + reduction[2]	1.9	3.6

[1]The activated preparation was a pineal homogenate treated as described in Fig. 1 with cystamine (pH 8.5; 1 mM for experiment I and 10 mM for experiment II). It was then subjected to the treatments listed.

[2]Reduction was by treating the preparation with DTT (10 mM) for 10 min at 37°.

[3]Gel filtration was accomplished by passing the preparation (200 µl) through a Sephadex G-25 column (18 x 0.5 cm) equilibrated with 0.1 M sodium phosphate buffer, pH 6.8 (flow rate 20 ml/h). This procedure completely separated free cystamine from macromolecules.

The observations supporting this are: first, that whereas cystamine is active, both the reduced form of cystamine, cysteamine, and the analogous diamine, diaminohexane, were inactive. This indicates that activation by cystamine requires the intact disulfide bond. Second, activation was enhanced at pH 8.5, as compared to pH 6.8. This is consistent with the hypothesis because the formation of the probable active form of the thiol participating in disulfide exchange, the thiolate ion (RS^-), would be favored at the higher pH. This would accelerate the rate of mixed disulfide formation (9,12). Third, the activation was reversed by treatment with DTT, which is capable of reducing a mixed disulfide formed by the interaction of cystamine with a protein SH group.

One observation which does not appear to fit with the model we propose is that GSH treatment activated acetyl-CoA hydrolase. This is in contrast to the effects of treatments with other thiols or with GS-SG, which either inhibited the enzyme or had no activating effect. One hypothetical explanation for this observation is that GSH first reacted with either a thiol or disulfide, resulting in the formation of a disulfide which activated the enzyme (9).

Some of the characteristics of cystamine-activation of acetyl-CoA hydrolase, including pH dependency and specificity, have previously been described for activation of another enzyme, fructose 1,6 diphosphatase (12). It should be pointed out that this was the first reported case of activation of an enzyme by disulfide exchange.

The observations presented in this report raise the interesting possibility that pineal acetyl-CoA hydrolase activity is physiologically regulated through disulfide exchange. It appears reasonable to pursue this because alterations of pineal acetyl-CoA hydrolase would have profound effects on energy metabolism in general and melatonin production in particular.

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