De Grandis (9) and Nagatomo and Aizawa (10) reported that SDH activity in inflamed tissues is increased. It is also known that the size of mitochondria in rat normal tissues varies (11). SDH activity in $600 \times g$ sup which contains all sizes of mitochondria was markedly increased by serotonin, but the activity in $12,000 \times g$ ppt was not changed. It was expected that SDH activity of unprecipitated mitochondria at $12,000 \times g$ (light mitochondria) would be changed by serotonin. But the increase of SDH activity by serotonin was not observed when it was measured in hypotonic medium which may swell and rupture mitochondria (12, 13). Therefore, the amount of SDH in mitochondria may be unchanged by serotonin (13–15). These results suggest that the mitochondrial membrane permeability for substrate, especially succinate, might change with local administration of serotonin. In the present experiment, it was demonstrated that intramuscular injection of serotonin increased SDH activity in the injected muscle just as was observed when dextran was used as the phlogistic (1). These data collectively indicate that an increase in SDH activity induced by serotonin injection is not specific for serotonin but may be related to the nature of "inflammatory response" in the rat.

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DIFFERENT PATTERNS OF INHIBITION OF DOPAMINE- β -HYDROXYLASE BY CYSTEINE

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Accepted December 18, 1975

It has previously been reported (1) that dopamine- β -hydroxylase [DBH: 3,4-dihydroxyphenethylamine, ascorbate- O_2 oxidoreductase (hydroxylating), EC 1.14.2.1] was inhibited

by sulfhydryl compounds such as cysteine, glutathione and coenzyme A and that the inhibition of DBH by these sulfhydryl compounds was restored by the addition of sulfhydryl type reacting reagents such as N-ethylmaleimide (NEM). However, when the partially purified dopamine- β -hydroxylase (PDBH) preparation was pretreated with cysteine, we found that the inhibition of DBH activity was not reversed by the addition of NEM or by a dialysis of the cysteine-PDBH preparation although the extent of the inhibition of DBH activity was dose dependent. In this note, we wish to report the inhibitory effect of cysteine on PDBH from the adrenal medulla of bovines.

DBH was purified from beef adrenal glands according to the procedure of Foldes et al. (2). In these studies the enzyme obtained at the stage of DEAE-cellulose chromatography elute was used. DBH activity was assayed by the conversion of tyramine to norysinephrine according to the procedure of Van der Schoot et al. (3). Reaction mixture contained in 1.0 ml: potassium phosphate buffer (pH 5.5) 100 μ moles, ascorbic acid 10 μ moles, fumaric acid 10 μ moles, tyramine hydrochloride 10 μ moles, catalase 200 Sigma unit, and the enzyme. The reaction mixture was preincubated at 37°C for 5 min. The incubation was started by the addition of substrate, and carried out for 15 min at 37°C in air. The reaction was terminated by adding 2 ml of 4 N ammonium hydroxide. The amounts of norysinephrine formed were determined by the conversion of norysinephrine to *p*-hydroxybenzaldehyde by adding periodate, followed by determination of this product by measuring the absorbance at 330 m μ . The formation of norysinephrine from tyramine under these conditions proceeded linearly for 20 min and was also proportional to the amount of enzyme used.

The effect of the inclusion of cysteine in enzymic reaction mixture in the absence and the presence of NEM is illustrated by Fig. 1A. It can be concluded from these data that the DBH activity was progressively reduced by increasing cysteine concentration in the absence of NEM, whereas these inhibitions were reversed by the addition of equimolar concentration

FIG. 1. (A): Effect of N-ethylmaleimide (NEM) on cysteine inhibition of dopamine- β -hydroxylase. Samples were preincubated for 5 min and incubated for 15 min. DBH activities were assayed in the absence and the presence of NEM at a concentration equivalent to the concentration of cysteine.

(B): Effect of N-ethylmaleimide (NEM) on cysteine-pretreated dopamine- β -hydroxylase. PDBH preparation was pretreated with cysteine of various concentrations for 10 min at 37°C. An aliquot was then measured for DBH activities in the absence and the presence of NEM at a concentration equivalent to the concentration of cysteine.

The term pCYSTEINE is defined as negative logarithm of molar concentration of cysteine.

of NEM. These results are in agreement with previous findings by Nagatsu et al. (1). However, when the PDBH preparation was pretreated with cysteine for 10 min at 37°C, and an aliquot of the mixture was assayed for enzymic activity in the absence and the presence of NEM, the restoration of the cysteine-induced inhibition of DBH was not observed by the addition of equimolar concentrations of NEM (Fig. 1B). As can be seen in Figs. 1A and 1B, NEM affected the cysteine inhibition of DBH in different ways. These results suggest that different mechanisms are responsible for the inhibitory effect of cysteine.

We also investigated the possibility of reversibility of the inhibition of cysteine-pretreatment DBH by removing cysteine by dialysis. The PDBH preparation was pretreated with cysteine for 10 min at 37°C, then dialyzed against 200 volumes of 0.05 M phosphate buffer, pH 6.5 at 4°C for 24 hr. The buffer was changed three times under dialysis, and then, an aliquot of the mixture was assayed for DBH activity in the presence of substrate in the range of 1.25–10 mM. The results are shown in Fig. 2. As can be seen, the decrease in DBH activity did not recover by dialysis. Since the observed K_m value (0.83 mM) for substrate is unaltered by the cysteine pretreatment (Fig. 2, inset), it seems likely that the mechanism responsible for the inhibition of DBH activity by cysteine pretreatment is not associated with the decrease of the affinity of the enzyme for substrate.

Inhibition of DBH by binding of the enzyme Cu has been suggested for several sulfur-containing compounds: diethyldithiocarbamate and disulfiram (4), thiourea derivatives (5, 6), dimethyldithiocarbamate (7), D-cysteine, L-cysteine, mercaptoethanol and coenzyme A (1). As shown above, the cysteine-pretreated inhibition of DBH was not reversed by the addition of NEM (Fig. 1B), by dialysis of the cysteine-PDBH preparation (Fig. 2) or by the addition of Cu^{++} (data not shown), suggesting that the mechanism of this inhibition of DBH by cysteine would not be through the binding of the Cu of DBH. From the present data and previously reported observations (1, 8), it might be reasonable to consider that

Fig. 2. Effect of dialysis of cysteine-pretreated dopamine- β -hydroxylase on kinetic parameter. PDBH preparation was pretreated with cysteine for 10 min at 37°C, and, after dialyzed the mixture, DBH activities were assayed in the presence of various concentrations of tyramine (final cysteine concentration, 10^{-4} M). Line Weaver-Burk plots (inset) of tyramine concentration against rate of hydroxylation with native (—○—) and cysteine-pretreated (—●—) enzymes. The velocity is expressed as μ moles of norepinephrine formed per 15 min.

the inactivation of DBH by cysteine in the absence of catalase is due to hydrogen peroxide (H_2O_2) rather than cysteine itself. The result in Fig. 2 also suggests that modification of the maximum velocity to tyramine may be simply due to the destruction of a part of active DBH. However, precise mechanism of the inhibition remains unknown.

In the presence of the naturally occurring inhibitors, DBH can become the rate-limiting enzyme of biosynthesis of norepinephrine (9). The purification and characterization of such inhibitors from various tissues of different species have been investigated in various laboratories (2, 10–15). In the process of studying the properties of the naturally occurring inhibitors of DBH (13, 14), we observed that the mechanism of inhibition of DBH by the naturally occurring inhibitors seems to be similar to that of the cysteine inhibition, and that these inhibitors seem to have sulfhydryl groups in the molecule. In view of the present finding and of wide distribution of the naturally occurring inhibitors in animal tissues (13, 14), it was suggested that these inhibitors might inhibit the DBH irreversibly *in vivo* and play an important role in the regulation of norepinephrine synthesis. Further investigation is underway to determine the mechanism of the irreversible inhibition of DBH by cysteine pretreatment.

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