Biochemical Pharmacology, Vol. 25, pp. 488–489, Pergamon Press, 1976. Printed in Great Britain

Irreversible inhibition of dopamine-β-hydroxylase by cysteine

(*Received* 14 *February* 1975; *accepted* 25 *July* 1975)

Several reports [1, 2]* have revealed the presence of natural inhibitors of dopamine-β-hydroxylase. The inhibition can be overcome by the addition of *N*-ethylmaleimide or Cu^{2+} to the incubation mixture, and this suggests that these endogenous inhibitors are sulfhydryl compounds. Previously, Nagatsu *et al.* [1] reported the effects of cysteine on dopamine-β-hydroxylase. Since the inhibition of dopamine-β-hydroxylase produced by cysteine [1] and 2-mercaptoethylguanidine [3] was reversed by dialysis or dilution of the mixtures, it has been postulated that these inhibitions may have been the result of a chelation *in situ* rather than a removal of the metal from the apoenzyme. We have found an irreversible inhibition of dopamine-β-hydroxylase by cysteine which was not restored by dialysis and dilution of the mixtures or by the addition of *N*-ethylmaleimide, which reacts with sulfhydryl groups [4]. Therefore, we have examined these inhibitory effects of cysteine on partially purified dopamine-β-hydroxylase in the present experiment.

Dopamine-β-hydroxylase was purified from beef adrenal glands according to the procedure of Foldes *et al.* [5]. In these studies, the enzyme obtained at the stage of DEAE-cellulose chromatography was used. Enzyme activity was assayed by following the conversion of tyramine to norsynephrine according to the procedure of Van der Schoot *et al.* [6]. The reaction mixture contained in 1·0 ml: potassium phosphate buffer (pH 5·5), 100 μmoles; ascorbic acid, 10 μmoles; fumaric acid, 10 μmoles; tyramine hydrochloride, 10 μmoles; catalase, 200 Sigma units; and the enzymes. The reaction was started by addition of the substrate and incubated for 15 min at 37° in air.

Partially purified dopamine-β-hydroxylase was preincubated with cysteine for 10 min at 37° in the absence and in the presence of catalase, after which the incubation mixtures were dialyzed against 10 mM phosphate buffer (pH 7·2), and aliquots of the mixtures were assayed for dopamine-β-hydroxylase activity (Table 1). Cysteine (10^{-4} M, final concentration) caused a reduction of dopamine-β-hydroxylase of about 60 per cent compared with control both in the absence and in the presence of catalase when assayed prior to the dialysis of the mixtures. After dialysis, the inhibitory effects of cysteine were abolished in the presence of catalase, in agreement with the work of Nagatsu *et al.* [1], but not in the absence of catalase, suggesting that cysteine inhibited dopamine-β-hydroxylase reversibly in the presence of catalase, but irreversibly in the absence of catalase. On the other hand, in agreement with a previously reported study [1], addition of cysteine to the dopamine-β-hydroxylase assay system resulted in inhibition of dopamine-β-hydroxylase activity, and equal concentrations of *N*-ethylmaleimide and cysteine completely prevented the cysteine inhibition of dopamine-β-hydroxylase.

Changes in the fractional inhibition of dopamine-β-hydroxylase by cysteine with dilution are shown in Table 2. Cysteine was preincubated with the enzyme for 20 min at 37°, after which 1:2 and 1:4 dilutions of the mixtures with medium were incubated for 15 min. The fractional inhibition was not significantly altered by the dilution when

Table 2. Effects of dilution of the cysteine–dopamine-β-hydroxylase mixture on fractional inhibition*

Dilution	Fractional inhibition
None	0·70
1:2	0·66
1:4	0·65

* Cysteine was preincubated with the partially purified dopamine-β-hydroxylase preparation for 20 min at 37°, after which time the mixture was diluted two or four times with medium substrate added and incubated for 15 min. The undiluted concentration of cysteine was 10^{-4} M. The term fractional inhibition (i) is defined as follows: $i = 1 - a = 1 - (v_1/v)$ where a is the fractional activity, v_1 is the inhibited velocity and v is the uninhibited velocity [7].

Table 1. Effects of dialysis on cysteine-pretreated dopamine-β-hydroxylase*

	Catalase absent (% of Control activity)		Catalase present (% of Control activity)	
	Before dialysis	After dialysis	Before dialysis	After dialysis
Control	100	94	100	60
Cysteine-pretreated	37	41	45	80

* Enzyme was preincubated with cysteine in the absence and in the presence of catalase (about 6000 Sigma units) for 10 min at 37°. One aliquot was then removed for assay of enzyme activities (final cysteine concentration, 10^{-4} M), and other aliquots were dialyzed against 1000 vol. of 10 mM phosphate buffer (pH 7·2) at 4° for 12 hr. Buffer was exchanged four times.

* H. Oyama, H. Izumi, S. Tuboi and H. Ozawa, manuscript submitted for publication.

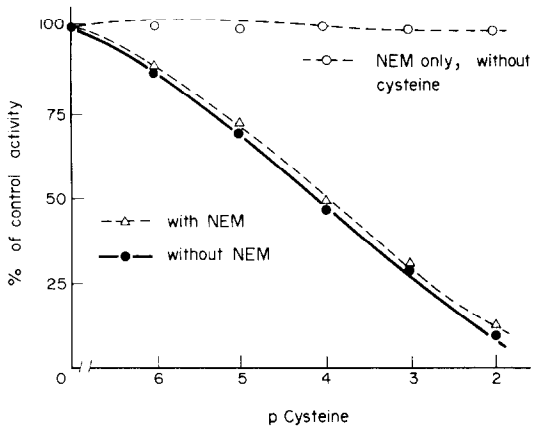

Fig. 1. Effects of *N*-ethylmaleimide (NEM) on cysteine-pretreated dopamine-β-hydroxylase. Enzymes were preincubated with various concentrations of cysteine for 10 min at 37°, after which the enzyme activities were assayed. Equal concentrations of NEM corresponding to concentrations of cysteine were added to each incubation medium. The term pCysteine is defined as follows: pCysteine = $-\log[\text{cysteine}]$, where [cysteine] is the molar concentration of cysteine.

compared with control, also indicating that cysteine inhibited dopamine-β-hydroxylase irreversibly. These results indicated that the mechanism responsible for the inhibition of dopamine-β-hydroxylase by cysteine might be somewhat dissimilar to that of 2-mercaptoethylguanidine [3].

In order to examine the effects of *N*-ethylmaleimide on dopamine-β-hydroxylase, *N*-ethylmaleimide was added to the incubation medium. The addition of *N*-ethylmaleimide did not affect the native and cysteine-pretreated enzyme activities at all, indicating that the inhibition of dopamine-β-hydroxylase by cysteine was not reversed by *N*-ethylmaleimide (Fig. 1). Furthermore, the cysteine-pretreated inhibition of dopamine-β-hydroxylase was neither restored by increasing the Cu^{2+} concentration nor by the addition of excess concentration of *N*-ethylmaleimide.

H_2O_2 is an inhibitor of dopamine-β-hydroxylase [8]. Consequently, catalase was added in all incubation mixtures in order to determine dopamine-β-hydroxylase activities. Recently, it has been reported [9] that catalase could have two functions: the prevention of the oxidation of dopamine-β-hydroxylase by endogenous peroxide compounds, and the prevention of oxidation of ascorbate which can occur when traces of metals are present. However, with our enzyme preparation, the preincubation of the enzyme alone for various times (up to 30 min) had no effects on enzyme activities, indicating that preincubation of the enzyme alone did not produce endogenous peroxide compounds.

The mechanism for the irreversible inhibition of dopamine-β-hydroxylase by cysteine may have been the result of: (1) formation of a chelate between cysteine and Cu^{2+}-dopamine-β-hydroxylase (this chelation is stable upon dialysis and dilution or after treatment with *N*-ethylmaleimide), or (2) formation of endogenous peroxide compounds by the interaction of dopamine-β-hydroxylase and cysteine. Considering the finding that the irreversible effects of cysteine on dopamine-β-hydroxylase occurred in the presence of inactivated catalase (obtained by exposure at 80° for 5 min, data not shown) but not in the presence of active catalase, it is more likely that this inhibition may be caused by the active form. However, since dopamine-β-hydroxylase is a copper metaloenzyme [10] and has been supposed to form a chelate with cysteine, this hypothesis should also be considered. In this case, catalase may act as an inhibitor on the formation of the chelate.

At present, the specific mechanism for this irreversible inhibition of dopamine-β-hydroxylase by cysteine is under investigation.

Pharmaceutical Institute,
Tohoku University,
Aobayama, Sendai 980, *Japan*

HIROSHI IZUMI
HIDEKO OYAMA
MAKOTO HAYAKARI
HIKARU OZAWA

REFERENCES

1. T. Nagatsu, H. Kuzuya and H. Hidaka, *Biochim. biophys. Acta* **139,** 319 (1967).
2. D. S. Duch and N. Kirshner, *Biochim. biophys. Acta* **236,** 628 (1971).
3. E. J. Diliberto, Jr., V. DiStefano and C. Smith, *Biochem. Pharmac.* **22,** 2961 (1973).
4. D. G. Smyth, A. Nagamatsu and J. S. Fruton, *J. Am. chem. Soc.* **82,** 4600 (1960).
5. A. Foldes, P. L. Jefferey, B. N. Preston and L. Austin, *Biochem. J.* **126,** 1209 (1972).
6. J. B. Van der Schoot, C. R. Creveling, T. Nagatsu and S. Udenfriend, *J. Pharmac. exp. Ther.* **141,** 74 (1963).
7. J. L. Webb, in *Enzymes and Metabolic Inhibitors*, Vol. 1, p. 52. Academic Press, New York (1963).
8. E. Y. Levin and S. Kaufman, *J. biol. Chem.* **236,** 2043 (1961).
9. D. Aunis, M. T. Miras-Portugal and P. Manel, *Biochim. biophys. Acta* **327,** 313 (1973).
10. S. Friedman and S. Kaufman, *J. biol. Chem.* **240,** 4763 (1965).