Biochemical Pharmacology, Vol. 30, pp. 171-174.

0006-2952/81/0115-0171$02.00/0

PRELIMINARY COMMUNICATIONS

ACTIVATION OF RAT BRAIN ADENYLATE CYCLASE BY COPPER PLUS DITHIOTHREITOL

Akemichi Baba, Tetsuroh Kihara, Eibai Lee and Heitaroh Iwata

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, Yamada-Kami, Suita, Osaka, Japan

(Received 2 September 1980; accepted 10 November 1980)

Cu(II), an indispensable metal ion for living cells, is known to oxidize SH groups to disulfide bonds in proteins (1). Many enzymes, such as adenylate cyclase (2-4), phosphodiesterase (5,6) and ATPase (7), are inactivated by fairly high concentrations of Cu(II). In studies on the mechanism of inactivation of adenylate cyclase by low concentrations of Cu(II), we found a new effect of this metal ion on the activity of the enzyme. This paper reports the activation of adenylate cyclase in the membrane fraction of rat brain by Cu(II) in the presence of dithiothreitol (DTT).

MATERIALS AND METHODS

Chemicals. [2,8-^{3}H]ATP (34.8 Ci/mmole) and [^{3}H-G]cyclic AMP (42.4 Ci/mmole) were obtained from the New England Nuclear Corp. (Boston, MA). All other chemicals were of analytical grade. Deionized, distilled water treated with Chelex-100 was used throughout.

Preparation of enzymes. Male Sprague-Dawley rats, weighing about 150-200 g, were decapitated. The cerebral cortex was immediately removed and homogenized with 9 vol. of 0.32 M sucrose in 2 mM Tris and 2 mM maleic acid, pH 7.4. The crude synaptic membrane fraction (M_1) was prepared by the method of De Robertis et al. (8). Adenylate cyclase was solubilized from the M_1 fraction with Lubrol-PX (9), in the absence of DTT. For comparison, liver plasma membranes (10), cardiac sarcolemma (11), and erythrocyte ghosts (12) were also prepared for assay of adenylate cyclase.

Assay of adenylate cyclase activity. The incubation medium for assay of adenylate cyclase contained 80 mM Tris-maleate (pH 7.4), 0.5 mM ATP, 2 mM $MgSO_4$, 1 mM 3-isobutyl-1-methylxanthine, and about 100 μg of protein of the M_1 fraction in a total volume of 500 μl. After preincubation for 5 min at 30°, the reaction was started by adding ATP and was carried out for 2.5 min at 30°. Heavy metal ions and DTT were added at zero time of preincubation. Cyclic AMP was assayed by the binding protein method as described previously (13). In some experiments, adenylate cyclase activity was also determined in the presence of ATP-regenerating system using [^{3}H]ATP (14), and [^{3}H]cyclic AMP was assayed by the method of Krishna et al. (15).

Protein was measured by the method of Lowry et al. (16). Cu(I) was determined with bathocuproine (17).

RESULTS AND DISCUSSION

The effects of various heavy metal ions on adenylate cyclase activity in the M_1 fraction were studied in the absence and presence of 10 mM DTT. In the absence of DTT, 10 μM Cu(II) and Zn(II) strongly inhibited the enzyme activity,

but at the same concentration Co(II), Ni(II), and Ba(II) had no effect. In contrast, simultaneous addition of Cu(II) and DTT caused 70 percent activation of the enzyme. The dose-response curves of the effects of Cu(II) are shown in the left panel of Fig. 1. Concentrations of 10-100 μM Cu(II) almost completely inhibited the enzyme activity in the absence of DTT, but caused about 70 percent activation in the presence of DTT. Inhibition and activation of the enzyme by Cu(II) were observed in the presence of an ATP-regenerating system, but no activation of the enzyme was observed when Cu(II) and DTT were added simultaneously with ATP at zero time of incubation (data not shown). At a concentration of 10 μM, Cu(II) caused activation of the enzyme even in the presence of 0.3 mM DTT, and maximum activation in the presence of 1-10 mM DTT (Fig. 1, right panel).

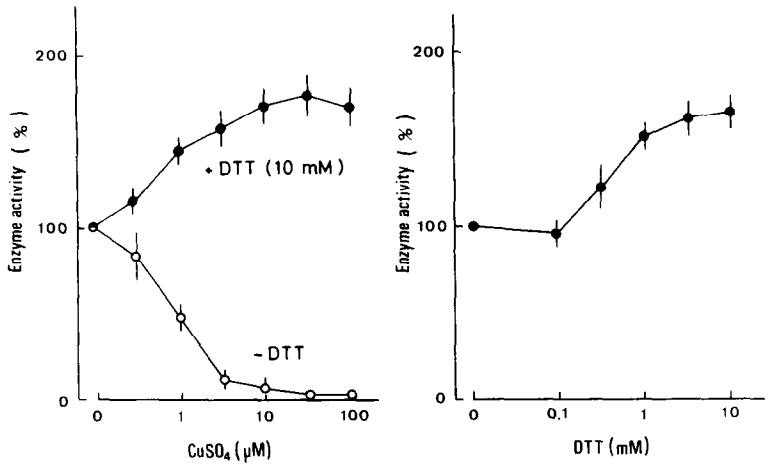

Fig. 1. Left panel: Effects of Cu(II) on adenylate cyclase activity of rat brain cortex M_1 fraction in the presence and absence of DTT. Points are means ± S.E. for six separate experiments. Right panel: Dose-response of the effect of DTT plus Cu(II) on adenylate cyclase of the M_1 fraction. Points are means ± S.E. for four separate experiments.

Cu(II) was found to lower the K_m value for ATP in the presence of DTT, but to elevate the K_m value in the absence of DTT (control, 50 μM; Cu(II), 110 μM Cu(II) + DTT, 14 μM). Activation by Cu(II) was not observed in the presence of glutathione, cysteine, or 2-mercaptoethanol. More than 95 percent of the Cu(II) was reduced to Cu(I) in the presence of DTT, indicating that DTT was oxidized during the incubation. We, therefore, prepared oxidized DTT (18), and examined its effect. This compound had no effect on the enzyme activity at concentrations of 1-1000 μM (data not shown).

As shown in Table 1, with our preparations of liver plasma membranes, cardiac sarcolemma, and erythrocyte ghosts, NaF-induced stimulation of adenylate cyclase was clearly observed. Futhermore, 1 μM glucagon caused about a 50-fold increase in the enzyme activity of liver plasma membranes, and 100 μM isoproterenol activated the enzyme in cardiac sarcolemma by 2-fold. However, unlike the brain enzyme, the enzyme in these preparations was not stimulated by Cu(II) (10 μM) plus DTT (5 mM). Solubilization of the brain adenylate cyclase with Lubrol-PX markedly increased the specific activity, but Cu(II) had

Table 1. Effects of various compounds on adenylate cyclase activity in various preparations

	Enzyme activity (pmole/mg/min)		
	Liver plasma membranes	Cardiac sarcolemma	Erythrocyte ghosts
Basal	2.7	21.0	2.6
Cu(II) (10 μM) + DTT (5 mM)	2.8	23.0	1.0
NaF (10 mM)	159.8	111.0	26.5
Glucagon (1 μM)*	103.6	N.D.**	N.D.**
Isoproterenol (100 μM)***	N.D.**	45.8	N.D.**

* In the presence of 100 μM GTP. ** Not determined.
*** In the presence of 10 μM guanyl-5'-yl imidodiphosphate.

no effect on this solubilized enzyme in either the presence or the absence of DTT (data not shown).

Cu(II) strongly inhibited adenylate cyclase activity in the membrane fraction of rat brain (IC_{50}, 1 μM). This result is in good accordance with a previous study (19). An interesting finding in the present study is that Cu(II), which alone is inhibitory, stimulated adenylate cyclase activity in the presence of DTT. There are two possible explanations for the mechanism of activation by Cu(II) plus DTT: (1) Cu(I), a reaction product, affected the enzyme directly, and (2) Cu(II) plus DTT activated the enzyme indirectly by modification of the membrane environment. Cu(II) was converted into Cu(I) by other SH compounds as well as DTT; however, these other SH compounds did not cause activation with Cu(II). Therefore, activation of the enzyme was not due to production of Cu(I). In the present study, we have no explanation for the lack of stimulatory effect of Cu(II) in the presence of other SH compounds on the enzyme activity. The lack of activation of Cu(II), however, may be due to redox potential differences, because redox potential of DTT is the most potent in various SH compounds. Recently, we found that Cu(II) evoked lipid peroxidation of the membrane if DTT was present in the medium (unpublished observation), and we are now studying the relation between lipid peroxidation of the membrane and the activity of adenylate cyclase.

The fact that the activation of adenylate cyclase by Cu(II) is specific for the brain enzyme seems interesting. The responses of adenylate cyclase in liver plasma membranes, cardiac sarcolemma, and erythrocyte membranes to various agents observed in the present study were similar to those reported previously (10,20,21), showing that the integrity of these membranes was maintained. At present, it is uncertain whether this specificity in the action of Cu(II) plus DTT is due to a difference in the properties of the membranes in these preparations, such as phospholipid composition.

REFERENCES

1. P. Hemmerich, in Biochemistry of Copper (Eds. J. Peisach, P. Aisen and W.E. Blumberg), p. 15. Academic Press, New York (1966).
2. R.A. Johnson and E.W. Sutherland, J. biol. Chem. 248, 5114 (1973).
3. K.G. Walton and R.J. Baldessarini, J. Neurochem. 27, 557 (1976).
4. L. Birnbaumer, S.L. Pohl and M. Rodbell, J. biol. Chem. 244, 3468 (1969).

5. W.Y. Cheung, Biochemistry 6, 1079 (1967).
6. T.E. Donnelly, Jr., Biochim. biophys. Acta 543, 273 (1978).
7. K. Bowler and C.J. Duncan, Biochim. biophys. Acta 196, 116 (1970).
8. E. De Robertis, C.R. De Lores Arnaiz, M. Alberrici, R.W. Butcher and E.W. Sutherland, J. biol. Chem. 242, 3487 (1967).
9. K. Sano, K. Nishikori, O. Noshiro and H. Maeno, Archs Biochem. Biophys. 197, 285 (1979).
10. P. Emmelot, C.J. Bos, E.L. Benedetti and P.H. Rümke, Biochim. biophys. Acta 90, 128 (1964).
11. P.V. Sulakhe, N.L. Leung and P.J. St. Louis, Can. J. Biochem. 54, 438 (1976).
12. J.T. Dodge, C. Mitchell and D.J. Hanahan, Archs Biochem. Biophys. 119, 124 (1963).
13. A. Baba, E. Lee, T. Matsuda, T. Kihara and H. Iwata, Biochem. biophys. Res. Commun. 85, 1204 (1978).
14. T.J. Lynch, E.A. Tallant and W.Y. Cheung, Archs Biochem. Biophys. 182, 124 (1977).
15. G. Krishna, B. Weiss and B.B. Brodie, J. Pharmac. exp. Ther. 163, 379 (1968).
16. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. 193, 265 (1951).
17. J.W. Landers and B. Zak, Am. J. clin. Path. 29, 590 (1958).
18. W.W. Cleland, Biochemistry 3, 480 (1964).
19. J.A. Nathanson and F.E. Bloom, Molec. Pharmac. 12, 390 (1976).
20. S.L. Pohl, L. Birnbaumer and M. Rodbell, J. biol. Chem. 246, 1849 (1971).
21. J.M. Richards and N.I. Swislocki, J. biol. Chem. 254, 6857 (1979).