ACTIVATION OF CEREBRAL GUANYLATE CYCLASE BY NITRIC OXIDE

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SUMMARY

Mouse cerebral guanylate cyclase was activated by catalase in the presence of sodium azide (NaN_3), which is known to form catalase-NO complex, while nitrosamines and nitric oxide (NO gas) were capable of activating cerebral guanylate cyclase in the absence of catalase. The activation of guanylate cyclase by NaN_3-catalase or nitrosamines was markedly inhibited by ferrohemoglobin which has a high affinity for NO, but not by ferrihemoglobin. These data suggest that NO or NO containing compounds may activate guanylate cyclase, whereas ferrohemoglobin may exhibit an inhibitory effect on the activation of guanylate cyclase, possibly by interacting with NO or NO containing compounds.

INTRODUCTION

Guanylate cyclase (GC), the enzyme that catalyzes the formation of guanosine 3', 5'-monophosphate (cGMP), is markedly activated by NaN_3 (1) and nitrosamines such as diethylnitrosamine (DEN) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (2). The activation of GC by NaN_3 requires a macromolecular factor (1, 3). We have found that this macromolecular guanylate cyclase activating factor (GAF) is identical to catalase and the hemolysate of erythrocyte, which contains catalase, does not activate GC in the presence of NaN_3 (4). This paper describes the stimulatory effects of nitric oxide (NO gas) and nitrosamines on GC activity, and the inhibitory properties of hemoglobin on the GC activation.

MATERIALS AND METHODS

Porcine blood was obtained from a local slaughter house. Cerebral cortices of male mice (STD-dd/Y) were homogenized with 20 volumes of 0.25 M sucrose using a glass homogenizer with a Teflon pestle. Catalase activity was measured and expressed as the values of K_{obs} (first-order reaction rate constant) according to the method of Bonnichsen (5, 6). Hemoglobin from porcine erythrocyte was partially purified by a continuous sucrose density gradient centrifugation (7). GC activity and GAF activity were measured as previously described (4). Beef liver catalase, cytochrome c, myoglobin and

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Table I

Effect of catalase on the activation of cerebral guanylate cyclase by NaN_3 and nitrosamines

Addition (2mM)	Guanylate Cyclase Activity (p moles cGMP synthesized/mg prot./min)	
	Catalase (-)	Catalase (+)
NaN_3	22 ± 2	255 ± 27
N-Methyl-N-Nitrosourea (MNU)	255 ± 10	255 ± 10
N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG)	250 ± 10	250 ± 11

Cerebral GC activity was assayed in the presence or absence of catalase (0.5 µg of protein). Each value in this table represents the mean ± S.D. obtained from four separate experiments.

methemoglobin were purchased from Sigma Chemical Co. [^{3}H] GTP (11ci/mmol) was purchased from Radiochemical Centre, Amersham. Nitric oxide (NO gas) was generated according to the method of Blanchard (8). Protein concentration was determined by the method of Lowry et al (9).

RESULTS

Nitrosamines are known as a potent activator of GC (2). We examined whether or not catalase is required for the activation of cerebral GC by nitrosamines. Table I shows that cerebral GC was activated by N-methyl-N-nitrosourea (MNU) and MNNG, nitrosamines having carcinogenic properties, in the absence of catalase. Other carcinogenic compounds such as urethan, methylcholanthrene, 4-nitroquinoline-1-oxide, 2-acetylaminofluorene had no stimulatory effect on cerebral GC in the presence or absence of catalase. Although the hemolysate of erythrocytes did not exhibit any GAF activity, GAF activity in the hemolysate appeared following the removal of hemoglobin by chloroform-ethanol treatment and increased by applying the further purification procedures for catalase according to the method of Bonnichsen (5)

Table II

Purification of guanylate cyclase activating factor (GAF) from porcine erythrocyte

Fraction	Total Protein (mg)	Total Catalase Activity ($K_{obs} \times 10^{-3}$)	Specific Activity: Catalase (K_{obs}/mg prot.)	Specific Activity: GAF (unit*/mg prot.)
1. Hemolysate	11,400	5.0	0.043	0
2. Chloroform-ethanol treatment	742	0.51	0.68	250
3. $(NH_4)_2SO_4$ fractionation (30-50%)	42	0.18	4.33	305
4. Acetate (pH 5.0) treatment	37.8	0.21	5.60	690
5. Acetone treatment	9.8	0.10	10.9	800

* One unit of GAF activity was arbitrarily defined as the amount which is required to give 50% stimulation of cerebral GC in the presence of 2mM NaN_3.

(Table II). When the purified catalase (acetone treated fraction; see Table II) was analyzed on a continuous sucrose density gradient centrifugation as previously described (4), it was found that the fraction having maximal catalase activity coincided with that of GAF activity. If partially purified hemoglobin was added to the reaction mixture, the activation of cerebral GC by NaN_3-catalase or nitrosamines was significantly attenuated. Half maximal inhibition of the NaN_3-catalase- and MNU-activated GC by hemoglobin was obtained at the hemoglobin concentrations of approximately 0.1mg/ml and 0.3mg/ml, respectively (Fig.1). Methemoglobin, cytochrome c and myoglobin had no inhibitory effect, but carbon monoxide-hemoglobin showed an inhibitory effect (Table III). When liver and cerebral homogenates were briefly exposed to nitric oxide (NO gas) and then GC activity was measured,

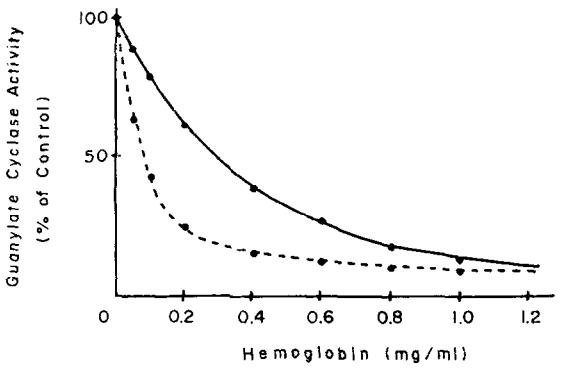

Fig. 1. Inhibition of NaN_3-catalase-activated and methylnitrosourea (MNU)-activated guanylate cyclase activities by hemoglobin:
Various concentrations of partially purified hemoglobin from porcine erythrocytes were mixed with cerebral homogenate and then GC activity in the cerebral homogenate was assayed in the presence of NaN_3-catalase or methylnitrosourea. ●——● Methylnitrosourea (2 mM). ●- - -● NaN_3 (2 mM) and catalase (0.5 µg of protein). One hundred percent was expressed as GC activities maximally stimulated by NaN_3-catalase or methylnitrosourea in the absence of hemoglobin.

Table III

Inhibition of NaN_3-catalase-activated guanylate cyclase activity by heme containing protein

Addition (0.5mg/ml)	Guanylate Cyclase Activity (p moles cGMP synthesized/mg prot./min)
None	250 ± 24
Hemoglobin *	40 ± 2
Methemoglobin*	255 ± 27
Methemolglobin (Sigma)	210 ± 20
CO-hemoglobin	60 ± 2
Cytochrome c	245 ± 21
Myoglobin	230 ± 21

Each heme containing protein was added to the cerebral homogenate and then GC activity was assayed in the presence of NaN_3 (2mM) and catalase (0.5 µg of protein).
* Hemoglobin (10 mg/ml) was oxidized to methemoglobin by adding $NaNO_2$ (1 mg/ml). $NaNO_2$ (50 µg/ml), which was included in the methemoglobin preparation (0.5 mg/ml), had no appreciable effect on cerebral GC activity. Each value in this table represents the mean ± S.D. obtained from four separate experiments.

Table IV

Activation of guanylate cyclase by nitric oxide (NO gas)

	Guanylate Cyclase Activity (p moles cGMP synthesized/mg prot./min)	
	Cerebrum#	Liver#
Control	21 ± 1	4 ± 1
Nitric oxide* (NO)	230 ± 30	85 ± 10

* Nitric oxide was generated in a Kipp's gas generator saturated with nitrogen as a following equation.

$$2NaNO_2 + 2FeSO_4 + 3H_2SO_4 \longrightarrow Fe_2(SO_4)_3 + 2NaHSO_4 + 2H_2O + \underline{2NO}$$

Each tissue homogenate was exposed to NO gas for 2 sec before assaying GC activity.
Each value in this table represents the mean ± S.D. obtained from four separate experiments.

the full activation of GC by nitric oxide was observed in both homogenate preparations (Table IV).

DISCCUSION

The gassing of nitric oxide alone significantly activated GC in the liver and cerebral homogenates. This indicates that catalase is not required for the activation of GC by nitric oxide, since cerebral homogenate contains little activity of catalase (4). Catalase inhibitors such as NaN_3 and NH_2OH activate cerebral GC in the presence of catalase. Although we first thought that conformational changes in catalase molecule by these inhibitors may be involved in the activation of GC (4), present results suggest that it is unlikely to be the case. Since nitrosamines and nitric oxide activated GC without catalase, and catalase-NO complex is known to be formed by catalase-NaN_3 or catalase-NH_2OH reaction (10), it is suggested that NO or NO containing compounds may activate GC. The hemolysate of

erythrocyte contains high activity of catalase (5), but did not activate cerebral GC in the presence of NaN_3. When hemoglobin was removed from the hemolysate of erythrocyte, the CAF activity became detectable in the hemolysate. Furthermore analysis of the hemolysate by a continuous sucrose density gradient centrifugation revealed that the CAF from erythrocytes is also identical to catalase. Ferrohemoglobin suppressed the activation of cerebral GC by NaN_3-catalase or nitrosamines. Ferrohemoglobin and ferrocytochrome a_3, but not ferrihemoglobin, are well known as compounds to form complex with NO (11, 12). These results also suggest that NO or NO containing compounds may be involved in the processes of GC activation by NaN_3-catalase or nitrosamines. Although it is possible that many agents which contain amine residue might be converted to NO or NO containing compounds in vivo by catalase or drug metabolizing enzymes such as hepatic microsomal P_{450}, physiological substances having similar properties as NO in terms of GC activation remain to be elucidated.

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