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CHARACTERIZATION OF CEREBELLAR GUANYLATE CYCLASE USING *N*-METHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE

PRESENCE OF TWO DIFFERENT TYPES OF GUANYLATE CYCLASE IN SOLUBLE AND PARTICULATE FRACTIONS

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Summary

Some characteristics of guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) in subcellular fractions prepared from rat cerebellum have been analyzed on the basis of responsiveness to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and inhibitors related to *N*-nitroso compounds.

The enzyme in 100 000 × *g* supernatant and crude mitochondrial (P_2) fractions were differently activated (11- and 2.5-fold, respectively) by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The soluble fraction obtained by hypo-osmotic treatment and subsequent recentrifugation of the P_2 (P_2-soluble) contained a significantly higher total guanylate cyclase activity than that of the starting material (P_2). The P_2-soluble fraction also exhibited a lower responsiveness (1.5-fold) to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine than that found in the P_2. The membrane fraction prepared from the P_2 (P_2-membrane) had no response to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Hemoglobin and vitamin A derivatives significantly inhibited both *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-activated 100 000 × *g* supernatant and basal P_2-soluble enzyme activities, without effect on the basal activities in 100 000 × *g* supernatant and P_2-membrane fractions.

The present results suggest that two different types of guanylate cyclase may be present in rat cerebellum in terms of the responsiveness of *N*-nitroso compounds, and P_2-soluble guanylate cyclase seems to be activated endogenously

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Abbreviations: cyclic GMP, guanosine 3',5'-monophosphate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

through a mechanism similar to the action of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Introduction

Guanylate cyclase (EC 4.6.1.2) catalyzes the formation of guanosine 3',5'-monophosphate (cyclic GMP), which is supposed to act as an intracellular messenger of the action of various hormones or neurotransmitters. Although it has been reported that various biologically-active compounds, including cholinergic agonists [1], depolarizing agents [2] and excitatory amino acids [3], induce the increase of cyclic GMP content in an intact cell system, they fail to activate guanylate cyclase in a cell-free system.

In recent years, it has been demonstrated that guanylate cyclase in a cell-free system is activated by compounds such as NaN_3 [4], nitrosamines [5] and NO [6,7] which is considered to form nitroxy free radicals. In neural tissues, Kimura et al. [4] reported that soluble enzymes from rat cerebral cortex and cerebellum are markedly activated by NaN_3 in the presence of a protein activator, while NaN_3 activation on particulate enzymes does not require the protein activator. This protein activator has been also found to be substitutive by catalase [8]. On the other hand, it has been reported that guanylate cyclase in rod outer segments from frog [9] and bovine [10] retinas is mostly membrane-bound and has no responsiveness to nitrosamines nor to NaN_3 plus catalase. These reports clearly indicate that the soluble and membrane-bound enzymes have different characteristics in terms of the response to NaN_3 or nitroso compounds.

The cerebellum is known to be a neuronal tissue having a significantly high content of cyclic GMP [11]. In addition, it has been demonstrated that in the cerebellum, particulate-bound guanylate cyclase activity is higher than that in the soluble fraction [12].

In the present study, we have attempted to clarify whether or not soluble and particulate-bound guanylate cyclases in rat cerebellum possess different characteristics in terms of their responsiveness to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), which is known to be one of the most potent guanylate cyclase activators among *N*-nitroso compounds, and to hemoglobin [7,14] and vitamin A derivatives [15] which are also known to suppress the activation of guanylate cyclase by *N*-nitroso compounds.

Materials and Methods

Chemicals. [8-^{3}H]GTP (spec. act., 14 Ci/mmol) and cyclic [8-^{3}H]GMP (spec. act., 21 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, U.K.). Major chemicals and enzymatic preparations used were obtained from the following sources, respectively: MNNG, sodium nitroprusside, diethyl nitrosamine and hydroxylamine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan), sodium azide and methylnitrosourea (Nakarai Chemicals Ltd., Kyoto, Japan), 3-isobutyl-1-methyl xanthine (Aldrich Chemical Co., Milwaukee, WI, U.S.A.), GTP, cyclic GMP, retinal, retinol, retinoic acid,

creatine phosphate, creatine kinase from rabbit muscle and catalase from beef liver (Sigma Chemical Co., St. Louis, MO, U.S.A.), and cyanmethemoglobin (BDH Chemicals Ltd., Poole, U.K.). Polyethylenimine cellulose and cellulose for thin-layer chromatography were purchased from the Merck Co. (Darmstadt, F.R.G.).

Subcellular fractionation of rat cerebellum. Male Wistar rats, weighing 200—250 g, were killed by decapitation and the cerebella were quickly removed and chilled on ice. All subsequent procedures were carried out at 0—4°C. The organs were homogenized with 9 vols. of 0.32 M sucrose in a glass homogenizer with Teflon pestle. Subcellular fractionation performed was essentially similar to the procedures described by Rodríguez de Lores Arnaiz et al. [16]. The primary fractionation was performed by successively centrifuging the homogenate to obtain nuclear (P_1; 900 × *g*, 10 min), crude mitochondrial (P_2; 11 500 × *g*, 20 min), microsomal (P_3; 100 000 × *g*, 60 min) and supernatant (S; 100 000 × *g*, 60 min, 'Cytosol') fractions. Subfractionation of the P_2 was carried out following a hypo-osmotic treatment: the P_2 was homogenized with ice-cold distilled water (10 ml per g wet tissue wt.) and the treated homogenate (fraction W) was centrifuged to obtain M_1 (20 000 × *g*, 30 min, pellet), M_2 (100 000 × *g*, 60 min, pellet) and M_3 (100 000 × *g*, 60 min, supernatant, 'P_2-soluble') fractions. To obtain membrane preparations from the P_2, the fraction W was directly centrifuged at 100 000 × *g* for 60 min to obtain the precipitate ($M_1 + M_2$ fraction) which was subsequently subjected to sonication procedures for 30 s using an ultrasonic disintegrator (Ohtake Sonicator Model 150, at force 4) in the medium containing 10 mM Tris-HCl (pH 7.7), 100 mM NaCl, 0.5 mM EDTA, and then centrifuged at 100 000 × *g* for 60 min to obtain the precipitate ('P_2-membrane': M_{1+2} fraction). All precipitates prepared were dissolved in distilled water. Each enzymatic assay was performed using freshly-prepared preparations.

Assay of guanylate cyclase activity. Guanylate cyclase activity was assayed in 20 μl of reaction mixture containing 50 mM Tris-HCl buffer (pH 7.7), 4 mM $MnCl_2$, 0.1 mM [^{3}H]GTP (0.5 Ci/mmol), 0.5 mM isobutyl methylxanthine, 10 mM theophylline, 3 mM cyclic GMP, 15 mM creatine phosphate, 20 μg creatine kinase and the enzyme sample. Enzyme activity was determined by incubating for 10 min at 37°C and terminated by the addition of 5 μl of 2 M acetic acid and boiling at 80°C for 2 min. After addition of 10 μl of 50 mM authentic cyclic GMP, 10-μl aliquots of the reaction mixture were subjected to thin-layer chromatography using poly(ethyleneimine) cellulose as described by Keirns et al. [17]. The radioactivity of the isolated cyclic [^{3}H]GMP was determined by liquid-scintillation spectrometry using a scintillation cocktail consisting of 0.3% (w/v) 2,5-diphenyloxazole, 0.02% (w/v) 1,4-bis[2-(5-phenyloxazolyl)]-benzene and 33% Triton X-100 in toluene. In order to estimate the degradation of cyclic GMP, cyclic [^{3}H]GMP was added to the complete reaction mixture, and the concentration of cyclic [^{3}H]GMP remaining after the incubation was determined using the same thin-layer chromatographic procedures. The amount of [^{3}H]GTP remaining at the end of the incubation period was also determined by isolating [^{3}H]GTP using thin-layer chromatography.

The radioactive cyclic GMP was produced lineally for at least 20 min under the standard assay conditions when 10—40 μg protein per assay were em-

ployed. The recovery of added cyclic [^{3}H]GMP was more than 90%, whereas [^{3}H]GTP remaining after the incubation was found to be more than 80% of the initial concentration.

Other methods. Partially-purified hemoglobin from rat blood was freshly prepared by a continuous sucrose density-gradient centrifugation [18]. The concentration of hemoglobin was determined by the cyanmethemoglobin method [19] using commercially-available cyanmethemoglobin as a standard. Catalase activity was measured and expressed as values of K_{obs} (first-order reaction rate constant) according to the method of Bonnichsen [20]. The protein concentration was determined by the method of Lowry et al. [21]. Statistical significance of the results was assessed using Student's *t*-test.

Results

Subcellular distribution of guanylate cyclase activity in rat cerebellum assayed in presence and absence of MNNG

Subcellular distribution of guanylate cyclase activity in rat cerebellum was examined in the presence and absence of MNNG (Table I). In the absence of MNNG, approx. 50 and 25% of the total activity were recovered in the P_2 and S fractions, respectively. On the other hand, more than one half of the total activity was detected in the S fraction when assayed in the presence of MNNG, since the enzyme in the S fraction was more markedly activated (11-fold) than those in other fractions. The enzyme in the P_2 fraction was also stimulated by 1 mM NaN_3 to the same extent (2.5-fold) as in the case of MNNG.

Since the P_2 fraction exhibited a high total guanylate cyclase activity, further fractionation of P_2 following hypo-osmotic treatments was performed. The distribution of guanylate cyclase in submitochondrial fractions is shown in Table II. Guanylate cyclase activity in the M_1 fraction, containing mostly membranes derived from synaptosomes, mitochondria and myelin, was activated only 1.2-fold by MNNG, whilst that in the M_2 fraction, containing mostly synaptic vesicles and some mebranes, had little or no responsiveness to MNNG.

TABLE I

SUBCELLULAR DISTRIBUTION OF GUANYLATE CYCLASE IN RAT CEREBELLUM

Guanylate cyclase activity in each fraction was assayed in the presence and absence of MNNG (1 mM). Each value represents the mean of three separate experiments.

Fraction	Protein (mg/g wet wt.)	Guanylate cyclase activity			
		Total activity (nmol/min per g wet wt.)		Specific activity (pmol/min per mg protein)	
		—MNNG	+MNNG	—MNNG	+MNNG
Homogenate	102.5	2.48	11.32	24.7	115.4
P_1	17.7	0.33	0.68	18.3	40.1
P_2	51.3	1.29	3.24	24.2	60.2
P_3	13.4	0.28	0.30	20.2	21.8
S	26.4	0.60	6.63	22.8	252.9
Recovery (%)	105.7	102.3	96.2	—	—

TABLE II

DISTRIBUTION OF GUANYLATE CYCLASE ACTIVITY IN SUBMITOCHONDRIAL FRACTIONS OBTAINED BY HYPO-OSMOTIC TREATMENTS

Guanylate cyclase activity in each fraction was assayed in the presence and absence of MNNG (1 mM). Each value represents the mean of three separate experiments.

Fraction	Protein (mg/g wet wt.)	Guanylate cyclase activity			
		Total activity (nmol/min per g wet wt.)		Specific activity (pmol/min per mg protein)	
		−MNNG	+MNNG	−MNNG	+MNNG
W	54.2	1.43	2.95	25.8	52.2
M_1	35.3	0.79	0.93	23.2	27.5
M_2	5.1	0.08	0.09	18.1	20.5
M_3	15.2	2.53	3.82	168.5	250.3
Recovery (%)	103.2	241.5	166.4	—	—

These observations suggest the possibility that MNNG-sensitive enzyme may be transferred to the P_2-soluble (M_3) fraction. It has been found, however, that the enzyme activity in the M_3 fraction is activated only 1.5-fold by MNNG, and total as well as specific activities in the M_3 fraction are higher than those found in the original fraction W, even in the absence of MNNG. These results are consistent with the observations reported by Deguchi et al. [22] that guanylate cyclase activity in the crude mitochondrial fraction from rat cerebral cortex is increased significantly after hypo-osmotic treatment. Since the M_1 and M_2 fractions behaved identically with regard to responsiveness to MNNG, the combined $M_1 + M_2$ fraction was used in following studies as the P_2-membrane (M_{1+2}) fraction without further separations.

When the M_3 and $M_1 + M_2$ fractions were combined, specific activity recovered to the same level observed in fraction W and responsiveness to MNNG also became similar to that of fraction W. The P_2-membrane (M_{1+2}) fraction, prepared from sonicated $M_1 + M_2$ fractions, exhibited a specific activity of 29.4 pmol/min per mg protein (the basal activity), and the recovery of total activity in this fraction was 102% compared with that in the $M_1 + M_2$ fraction.

Responsiveness to the activators

Effects of MNNG, NaN_3 and NaN_3 plus catalase on guanylate cyclase activities in 'cytosol', 'P_2-soluble' and 'P_2-membrane' fractions are shown in Table III. MNNG induced a marked activation of guanylate cyclase in the cytosol fraction, but showed relatively small activation in the P_2-soluble enzyme. No stimulation was observed in the case of the P_2-membrane enzyme. Although the enzyme activities in cytosol and P_2-membrane fractions measured in the absence of MNNG increased lineally at least up to 40 min, MNNG-activated cytosol and P_2-soluble enzymes lost the lineality at points of 30 and 20 min, respectively. The effect of MNNG on the cytosol guanylate cyclase was dependent upon its concentration up to 0.5 mM, and the concentration that produced the half-maximal activation was 0.1 mM (Fig. 1). The basal

TABLE III

EFFECT OF MNNG, NaN_3 AND NaN_3 PLUS CATALASE ON RAT CEREBELLAR GUANYLATE CYCLASE

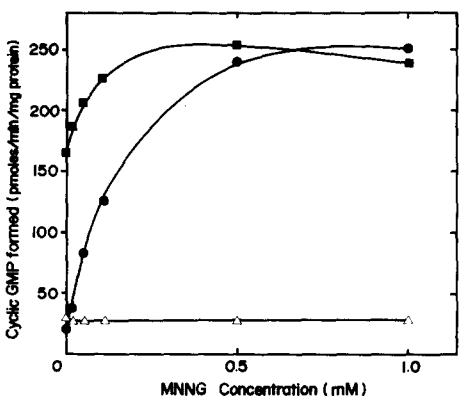
Guanylate cyclase activity in each preparation was assayed in the absence (control) and presence of MNNG (1 mM), NaN_3 (1 mM) or NaN_3 (1 mM) plus catalase (1 μg). Each value represents the mean ± S.E. obtained from four separate experiments.

Compound	Guanylate cyclase activity (pmol/min per mg protein)		
	Cytosol (S)	P_2-soluble (M_3)	P_2-membrane (M_{1+2})
Control	24 ± 1.5	182 ± 12.3	28 ± 3.2
MNNG	301 ± 28.4 *	280 ± 19.5 *	29 ± 4.0
NaN_3	28 ± 3.2	278 ± 18.1 *	30 ± 2.9
NaN_3 + catalase	310 ± 33.5 *	283 ± 20.3 *	29 ± 4.2

* Significant at $P < 0.01$, compared to corresponding control value.

activity level of P_2-soluble enzyme (measured in the absence of MNNG) was significantly higher than that of the cytosol enzyme, whereas the extent of MNNG activation on P_2-soluble enzyme was rather small and reached almost the same level as that of cytosol enzyme.

The above results suggest the possibility that the P_2-soluble guanylate cyclase may be activated endogenously through a similar mechanism to the activation by MNNG. On the contrary, 1 mM NaN_3 activated the enzyme in the P_2-soluble fraction to the same extent as MNNG (1.5-fold) but not in the cytosol and P_2-membrane fractions (Table III). Since previous studies from our laboratory indicated that catalase is able to activate soluble guanylate cyclase in the presence of NaN_3 [8], purified catalase was added to the reaction mixture in



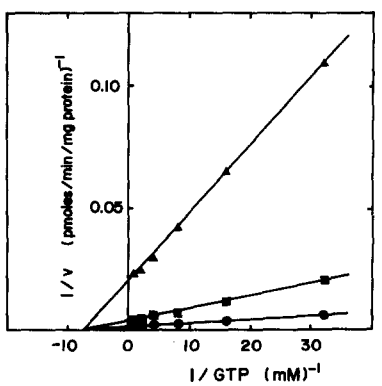


Fig. 1. Effect of MNNG on rat cerebellar guanylate cyclase. Guanylate cyclase activities in cytosol (S) (●), P_2-soluble (M_3) (■) and P_2-membrane (M_{1+2}) (△) fractions were assayed in the presence of various concentrations of MNNG. Each point represents the mean of three separate experiments.

Fig. 2. Double reciprocal plots of the kinetics for GTP of soluble guanylate cyclase of rat cerebellum. Kinetics for GTP of guanylate cyclase in the cytosol (S) fraction, in the presence (●) or absence (▲) of 1 mM MNNG, and the P_2-soluble (M_3) fraction (■) were determined. The standard assay conditions were employed except for varying GTP concentration.

the presence of NaN_3. Combined use of NaN_3 and catalase resulted in a marked activation of guanylate cyclase in the cytosol fraction but not in the P_2-membrane fraction, while no additive effect of catalase was observed in the P_2-soluble fraction compared to NaN_3 alone. To clarify possible causes of the differences between cytosol and P_2-soluble enzymes on the responsiveness to NaN_3, endogenous catalase activity was determined in these preparations. The P_2-soluble fraction contained catalase activity of $6.0 \cdot 10^{-3}$, expressed as K_{obs}/mg protein, while no activity was detected in cytosol and P_2-membrane fractions. These results indicate that NaN_3 activates the 'P_2-soluble' guanylate cyclase with the aid of catalase which is present endogenously in the P_2-soluble fraction. Other well known guanylate cyclase activators such as *N*-methyl nitrosourea, diethyl nitrosamine [5], and sodium nitroprusside [6] also activated in the same manner as MNNG, but these compounds were less potent than MNNG. On the other hand, hydroxylamine, which is capable of activating guanylate cyclase in the presence of protein activator or catalase [8,23,24], exhibited the same type of activation on the P_2-soluble enzyme as NaN_3, but this compound was also less potent than NaN_3.

Kinetics for GTP of guanylate cyclase activity

Fig. 2 shows the kinetics for GTP of the soluble enzymes. The P_2-soluble, cytosol and MNNG-activated cytosol enzymes showed classical Michaelis-Menten kinetics with an apparent K_m value of 0.13 mM, and V values calculated were 256, 49 and 830 pmol/min per mg protein, respectively. The P_2-soluble enzyme resembled MNNG-activated cytosol enzyme in the kinetics, since both enzymes exhibited Michaelis-Menten kinetics having the same K_m

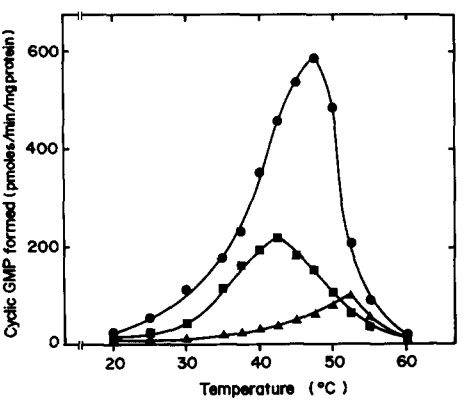


Fig. 3. Effect of temperature on soluble guanylate cyclase activities in rat cerebellum. Guanylate cyclase activities in the cytosol (S) fraction, in the presence (●) or absence (▲) of 1 mM MNNG, and the P_2-soluble (M_3) fraction (■) were measured at various temperatures as indicated. The protein contents in cytosol (S) and P_2-soluble (M_3) fractions were 25.2 and 4.3 μg/assay, respectively. The standard assay conditions were used except that the incubation time was altered to 5 min and the temperature for incubation was varied as indicated.

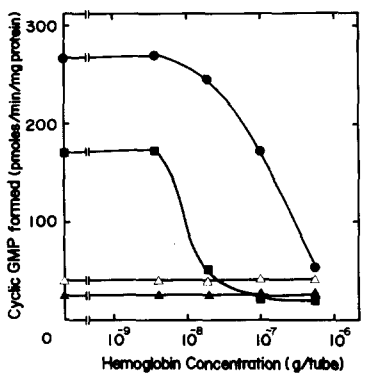


Fig. 4. Effect of hemoglobin on guanylate cyclase activity of rat cerebellum. Guanylate cyclase activities in cytosol (S), P_2-soluble (M_3) (■) and P_2-membrane (M_{1+2}) (△) fractions were assayed in the presence of various concentrations of hemoglobin. The enzyme activity in the cytosol (S) fraction was measured in the presence (●) or absence (▲) of 1 mM MNNG.

value with a relatively high V value. On the other hand, kinetics of the P_2-membrane enzyme did not exhibit Michaelis-Menten kinetics but showed a sigmoidal curve.

Effect of temperature

Guanylate cyclase activity in each soluble enzyme preparation was measured at various temperature (Fig. 3). Although the optimal temperature for the cytosol enzyme was 52.5°C, it was shifted to 47.5°C when activated by MNNG. The P_2-soluble enzyme had a lower optimal temperature of 42.5°C. When the effect of temperature on the P_2-membrane enzyme was examined, the temperature-activity curve did not show a typical bell-shaped curve as shown in the case of soluble enzymes and exhibited the maximal enzyme activity at 35 and 45°C. In addition, 1 mM MNNG had no influence on the P_2-membrane enzyme at various temperatures examined.

Effects of hemoglobin and vitamin A derivatives

Since hemoglobin is known to be a suppressor of guanylate cyclase activated by nitroso compounds [7,14], the effect of hemoglobin on guanylate cyclase was examined (Fig. 4). Dose-dependent inhibition by hemoglobin was observed in MNNG-activated cytosol enzyme but not in basal (non-activated) cytosol enzyme. On the contrary, the basal activity of P_2-soluble enzyme was markedly suppressed by hemoglobin. Guanylate cyclase in the P_2-membrane preparation was not affected at all by hemoglobin.

The effect of vitamin A derivatives, which are known to be inhibitors on nitroso compound-activated guanylate cyclase [15], was also examined (Table IV). In the same manner as hemoglobin, vitamin A derivatives also markedly inhibited the MNNG-activated cytosol enzyme activity as well as basal activity of the P_2-soluble enzyme, whilst no significant change of the activity in either basal cytosol or P_2-membrane fractions was observed. These results also suggest

TABLE IV

EFFECT OF VITAMIN A DERIVATIVES ON RAT CEREBELLAR GUANYLATE CYCLASE ACTIVITY

Each retinoid compound was dissolved in dimethylsulfoxide (DMSO) and added to the enzyme preparation. The final concentration of DMSO was 0.5% which had no significant effect on the enzyme activity. Guanylate cyclase activity in the cytosol fraction was assayed in the presence and absence of 1 mM MNNG. Each value represents the mean ± S.E. of three separate experiments.

Compound (1 mM)	Guanylate cyclase activity (pmol/min per mg protein)			
	Cytosol (S)		P_2-soluble (M_3)	P_2-membrane (M_{1+2})
	—MNNG	+MNNG		
Control	24 ± 2.1	297 ± 28.4	179 ± 12.7	31 ± 4.1
Retinal	25 ± 2.8	81 ± 12.0 *	17 ± 1.9 *	22 ± 2.3
Retinol	23 ± 2.7	43 ± 5.6 *	9 ± 0.6 *	25 ± 1.8
Retinoic acid	27 ± 3.0	77 ± 8.2 *	11 ± 1.2 *	27 ± 2.4

* Significant at $P < 0.001$, compared to corresponding control value.

that the P_2-soluble guanylate cyclase may be endogenously activated through a similar activation mechanism to that of the action of nitroso compounds.

Discussion

Kimura et al. [4] reported that guanylate cyclase from rat liver was markedly activated by NaN_3. This activation, however, required a protein activator, which was found to be substitutive with catalase [8]. On the other hand, it has been reported that nitrosamines [5], which contain a nitroxy group within their structure, and NO [6,7] activate guanylate cyclase in the absence of catalase. Since azide is known to be converted to reactive NO by catalase in the presence of H_2O_2 [25], possible involvement of free nitroxy radicals formed from NaN_3 in the activation of guanylate cyclase is also suggested. Furthermore, this activation was found to be prevented by hemoglobin [7,14] and vitamin A derivatives [15]. Considering that hemoglobin strongly interacts with NO [26,27] and vitamin A derivatives serve as a radical scavenger [28,29], these observations may also be regarded as an indication that guanylate cyclase has responsiveness to the nitroxy radical.

In the neural tissues, it was reported for the first time [4] that NaN_3 itself does not activate soluble guanylate cyclase, but markedly stimulates the particulate enzyme. It has been found recently, however, that soluble guanylate cyclase from the cerebral cortex is activated by NaN_3 in the presence of protein activator from liver [4] or catalase [8]. Furthermore, purified guanylate cyclase from synaptosomal soluble fractions of the cerebral cortex has been found to be activated by NaN_3 plus catalase as well as MNNG [30]. In the present study, we have examined the mechanism of activation by NaN_3 using the subfractions of crude mitochondrial (P_2) fraction obtained from rat cerebellum. According to our results, the mechanism would be explained as follows: NaN_3 is converted to reactive NO in the presence of catalase which is present endogenously in the P_2-soluble fraction, and the NO-catalase complex formed can activate 'soluble' guanylate cyclase in the P_2 fraction, whilst the enzyme in the P_2-membrane is not affected at all. It has been well established that the P_2 fraction from cerebral cortex as well as cerebellum contains a significant amount of synaptosomes, which possess a high activity of soluble guanylate cyclase [22]. Therefore, it is more likely that the responsiveness of the crude mitochondrial (P_2) fraction to NaN_3 may be due to the activation of synaptosomal soluble guanylate cyclase. Although the P_2-membrane-bound guanylate cyclase has various different characteristics to those of soluble enzymes as shown in this study, the most distinct difference must be in the difference of responsiveness to nitroso compounds. Considering the evidence that membrane-bound guanylate cyclase from frog rod outer segments is also not activated by nitroso compounds [9], it may be reasonable to conclude that membrane-bound guanylate cyclase from neural tissues generally has no responsiveness to nitroso compounds.

The P_2-soluble guanylate cyclase had a low responsiveness to MNNG as well as high basal activity which was suppressed markedly by hemoglobin and vitamin A derivatives. These characteristics resemble those of the MNNG-activated cytosol enzyme. This similarity was also found in kinetic behavior for

GTP as well as in the optimal temperature for the enzyme reaction. These results suggest that the P_2-soluble enzyme may be activated endogenously through a similar mechanism to that of the action of nitroso compound. Since the activation was latent in both P_2 and W fractions which contain membrane fractions, the possible regulatory roles of membraneous components in the activation of guanylate cyclase are also suspected. Deguchi [31] reported that guanylate cyclase in synaptosomal-soluble fraction from rat brain excluding the cerebellum is activated by an endogenous activator from the same fraction. It is presently unknown whether or not the P_2-soluble fraction from rat cerebellum is activated by the same endogenous activator as he reported. Although detailed mechanisms underlying the activation by an endogenous activator are unclear at present, it is noteworthy that guanylate cyclase in crude mitochondrial (P_2) soluble fraction is activated endogenously through a similar mechanism to that of the action of nitroso compounds. Considering the well known facts that the P_2 fraction contains most of the synaptosomes and that the P_2-soluble fraction obtained by hypo-osmotic treatment of P_2 mostly derives from the cytoplasm of synaptosomes, possible involvement of the endogenous guanylate cyclase activating factor in the physiological regulation of cyclic GMP at synapses may be suggested.

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