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EFFECTS OF ARGININE DERIVATIVES ON SOLUBLE GUANYLATE CYCLASE FROM NEUROBLASTOMA N1E 115 CELLS

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Abstract—The effects of L-arginine (Arg) derivatives on soluble guanylate cyclase from neuroblastoma N1E 115 cells were examined. The Arg derivatives were modified at the —NH_2, —COOH, Cα-proton or guanidino group of Arg. Among the synthesized derivatives, eight compounds, i.e. the 5-(dimethylamino)-1-naphthalenesulfonyl (DNS) ones, especially *N*-cyclohexyl-2-(*N*-DNSamino)-5-guanidino-2-methylvaleramide and 1-[2-(*N*-DNSamino)-2-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl)acetyl]-piperidine, were found to inhibit the activity of crude guanylate cyclase in the 105,000 *g* supernatant fraction of the cell homogenate. The enzyme, partially purified by a column of Chelex 100 Na^+, was also inhibited by these eight compounds. The mode of the inhibition was competitive. The K_i values were in the range of 2–8 μM for the enzyme in the 105,000 *g* supernatant fraction and 3–16 μM for the partially purified enzyme, in the presence of Mg^{2+} as a metal cofactor. In contrast, a new derivative, methyl 2-amino-5-guanidinovalerate (M Arg ME), as well as the Arg methyl ester (Arg ME) and Arg, were found to enhance the activity of the partially purified guanylate cyclase; K_A values of M Arg ME, Arg ME and Arg were approximately 9, 4 and 3 μM respectively. From these results, the free guanidino group including 2-imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl or 2-imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl and modification of the —NH_2 residue with a hydrophobic group such as DNS seemed to be essential for inhibition of the guanylate cyclase; however, the guanidino and —NH_2 residue of Arg should be free for activation by these Arg derivatives.

Cholinergic and α-adrenergic agonists and some hormones have been known to elevate cyclic GMP (cGMP) accumulation in various tissues and cultured cells [1–7]. By using a specific monoclonal antibody to soluble guanylate cyclase from rat brain [8], the guanylate cyclase has been shown to be localized within postsynaptic components (perikaryon and dendrites) in neurons but not within presynaptic terminals [9]. The study suggested that cGMP and guanylate cyclase may be related to postsynaptic events such as neuronal transmission and other intracellular processes. As in cell-free systems where acetylcholine and other transmitters fail to activate guanylate cyclase (EC 4.6.1.2), which catalyzes the formation of cGMP from GTP, the mechanism of the agonist-evoked cGMP augmentation in intact tissues remains to be elucidated [10, 11].

Many agents such as non-ionic detergents [11–14], nitroso compounds [15–18], lipids [19–22], heme-like substances [23–26], nitric oxide [18, 24, 27, 28] and endothelium-derived relaxing factor [29–34] have been reported to activate guanylate cyclases. On the other hand, a number of substances such as lysolecithin, methylhydroxylamine, trifluoroperazin [35], methylene blue and hemoglobin [24, 36, 37] have been shown to inhibit the activation of guanylate cyclase by nitroso compounds and other activators. The mechanism of both activators and inhibitors on guanylate cyclase still remains unknown.

Deguchi and coworkers [10, 14] have reported that synaptosomes of rat brain and 105,000 *g* supernatant fraction of neuroblastoma N1E 115 cells contain endogenous activators for soluble guanylate cyclase. One of the activators found in the acid extract of the supernatant fraction of rat brain was identified as L-arginine (Arg) by Deguchi and Yoshioka [38]. They also demonstrated that homoarginine, arginine methyl ester (Arg ME) and peptides such as tuftsin and bradykinin activate the soluble guanylate cyclase from neuroblastoma cells, but D-arginine, lysine, citrulline and peptides such as angiotensin-I and neurotensin do not [38]. No information is available concerning the activation mechanism of the guanylate cyclase by Arg and Arg derivatives.

Arg is known to activate partially purified soluble guanylate cyclase not only from neuroblastoma N1E 115 cells but also from synaptosomes of rat brain [38]. It is of interest to know whether endogenous Arg participates in the neuronal transmission via regulation of soluble guanylate cyclase. To study the biological significance of Arg, which may activate the guanylate cyclase, a potent inhibitor for the

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guanylate cyclase is required. In this study, we examined the effects of eighteen newly synthesized Arg derivatives and others on the soluble guanylate cyclase from neuroblastoma cells to gain insight into the structure–activity relationship of the derivatives.

MATERIALS AND METHODS

Materials. *p*-Toluenesulfonyl and 5-(dimethylamino)-1-naphthalenesulfonyl groups are abbreviated as tosyl and DNS respectively. The following compounds were synthesized in our laboratory [39, 40], and their structures are shown in Table 1: methyl 2-amino-5-guanidinovalerate hydrochloride (M Arg ME; Anal. calcd. for $C_8H_{19}N_4O_2 \cdot HCl$: C, 40.25; H, 8.02; N, 23.47. Found: C, 39.85; H, 7.81; N, 23.00); methyl 5-guanidino-2-methyl-2-(tosylamino)valerate hydrochloride (Anal. calcd. for $C_{15}H_{25}N_4O_4 \cdot HCl$: C, 45.85; H, 6.41; N, 14.26. Found: C, 45.24; H, 6.30; N, 14.09); methyl 5-guanidino-2-methyl-2-[*N*-(naphthalene-1-sulfonyl)-amino]valerate hydrochloride (Anal. calcd. for $C_{18}H_{24}N_4O_4S \cdot HCl \cdot H_2O$: C, 48.15; H, 6.06; N, 12.48. Found: C, 48.18; H, 5.65; N, 12.50); 5-guanidino-2-methyl-2-(*N*-tosylamino)valeric acid (uninvestigated); 2-(*N*-DNSamino)-5-guanidino-2-methyl valeric acid (Anal. calcd. for $C_{19}H_{27}N_5O_4S \cdot 1/2H_2O$: C, 53.00; H, 6.55; N, 16.27. Found: C, 52.66; H, 6.78; N, 17.04); propyl 2-(*N*-DNSamino)-5-guanidino-2-methylvalerate dihydrochloride (Anal. calcd. for $C_{22}H_{33}N_5O_4S \cdot 2HCl$: C, 49.24; H, 6.57; N, 13.05. Found: C, 49.43; H, 6.80; N, 12.36); *N*-cyclohexyl-2-(*N*-DNSamino)-5-guanidino-2-methylvaleramide dihydrochloride (Anal. calcd. for $C_{25}H_{38}N_6O_3S \cdot 2HCl \cdot H_2O$: C, 50.58; H, 7.13; N, 14.15. Found: C, 49.90; H, 7.09; N, 13.81); 2-amino-2-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl)acetic acid hydrochloride (Anal. calcd. for $C_6H_{12}N_4O_2 \cdot HCl$: C, 47.84; H, 5.56; N, 17.17. Found: C, 47.61; H, 5.49; N, 17.12); propyl-2-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl)-2-(tosylamino)-acetate hydrochloride (Anal. calcd. for $C_{16}H_{24}N_4O_4S \cdot HCl$: C, 47.46; H, 6.22; N, 13.84. Found: C, 47.13; H, 6.24; N, 13.17); 1-[2-(*N*-DNSamino)-2-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl)acetyl]piperidine dihydrochloride (Anal. calcd. for $C_{23}H_{32}N_6O_3S \cdot 2HCl \cdot 3/2H_2O$: C, 55.29; H, 7.06; N, 16.82. Found: C, 55.53; H, 6.88; N, 16.40); 2-amino-3-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl)propionic acid (uninvestigated); *N*-DNS-3-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl)alanine butyl ester dihydrochloride (Anal. calcd. for $C_{23}H_{33}N_5O_4S \cdot 2HCl$: C, 50.36; H, 6.43; N, 12.77. Found: C, 50.18; H, 6.56; N, 12.45); methyl (2*S*)-1-[2-(*N*-DNSamino)-3-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl)propionyl]-2-pyrrolidine-carboxylate dihydrochloride (Anal. calcd. for $C_{25}H_{34}H_6O_5S \cdot 2HCl \cdot 2H_2O$: C, 46.95; H, 6.30; N, 13.14. Found: C, 47.02; H, 5.91; N, 13.17); methyl 1-[2-(*N*-DNSamino)-3-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl)propionyl]piperidine-2-carboxylate dihydrochloride (Anal. calcd. for $C_{26}H_{36}N_6O_5S \cdot 2HCl \cdot 2H_2O$: C, 47.78; H, 6.48; N, 12.86. Found: C, 47.34; H, 6.30; N, 12.76); 1-[2-(*N*-DNSamino)-3-(2-imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl)propionyl]-4-methylpiperidine dihydrochloride (Anal. calcd. for $C_{25}H_{36}N_6O_3S \cdot 2HCl \cdot H_2O$: C, 50.76; H, 6.82; N, 14.21. Found: C, 50.66; H, 6.73; N, 13.88); 1-[2-(*N*-DNSamino)-3-(2-(*N*-nitro)imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl)propionyl]-4-methylpiperidine (Anal. calcd. for $C_{25}H_{35}N_7OS \cdot 1/2H_2O$: C, 54.14; H, 6.54; N, 17.68. Found: C, 53.99; H, 6.53; N, 17.57); 4-[2-(*N*-nitro)imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl]-2-phenyloxazol-5-yl-2,2-dimethylpropionate (Anal. calcd. for $C_{18}H_{21}N_5O_5$: C, 55.81; H, 5.46; N, 18.08. Found: C, 55.63; H, 5.19; N, 18.20); and 4-[2-(*N*-nitro)imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl]-2-(4-chlorophenyl)oxazol-5-yl-2,2-dimethylpropionate (Anal. calcd. for $C_{18}H_{20}ClN_5O_5$: C, 51.25; H, 4.78; N, 16.60. Found: C, 51.46; H, 4.78; N, 16.52). L-Arginine hydrochloride was purchased from the Nakarai Tesque Co. (Kyoto, Japan). *Nα*-Tosyl-L-arginine, *Nα*-benzoyl-L-arginine, *Nα*-tosyl-L-arginine methyl ester (TAME), N^g-tosyl-L-arginine (N^g-tosyl-Arg), leupeptine and agmatine were obtained from the Peptide Institute Inc. (Osaka, Japan). DNS-L-arginine (DNS-Arg) was from the Seikagaku Kogyo Co. (Tokyo, Japan). L-Arginine methyl ester dihydrochloride (Arg ME), L-argininamide dihydrochloride (Argamide) and N*α*-benzoyl-L-arginine methyl ester were purchased from the Sigma Chemical Co. (St Louis, MO). [8-^{3}H]GTP (12.6 Ci/mmol) was obtained from The Radiochemical Centre (Amersham, U.K.). GTP and cGMP were donated by the Yamasa Shoyu Co. (Chiba, Japan). Other chemicals were obtained from commercial sources.

Cell culture. Neuroblastoma N1E 115 cells, donated by Dr Takehiko Amano, Mitsubishi-Kasei Institute of Life Sciences, were grown in 90-mm Nunc plastic dishes in 12 ml of Dulbecco–Vogt modified Eagle's minimal essential medium (DMEM) supplemented with 8% fetal calf serum (Whittaker M.A. Bioproducts, U.S.A.) in a humidified atmosphere of 10% CO_2 and 90% air at 37°. The medium was changed every day, and cells were usually grown for 3–4 days. The confluent cells were collected and stored at −80° in DMEM containing 20% fetal calf serum and 10% dimethyl sulfoxide (DMSO) until use.

Preparation of guanylate cyclase. The frozen cells were thawed and then centrifuged at 200 *g* for 4 min. After washing with phosphate-buffered saline (0.14 M NaCl, 0.003 M KCl and 0.01 M phosphate buffer, pH 7.4), the cells were suspended in approximately 2 vol. of 5 mM Tris–HCl, pH 7.6, and homogenized. The homogenate was centrifuged at 105,000 *g* for 45 min. The supernatant fraction was used as a crude enzymic preparation of the guanylate cyclase. The supernatant fraction (5–7 ml) was further passed through a Chelex 100 Na^+ column (1 × 12 cm) to remove cations and eluted with distilled water at 4°. The guanylate cyclase activity was found in an effluent.

Assay of guanylate cyclase. The activity was assayed as described by Deguchi *et al.* [14]. The reaction mixture in a total reaction volume of 150 μl contained 7.5 μmol of Tris–HCl, pH 7.6, 0.75 μmol of creatine phosphate, 0.5 μmol of cGMP, 0.45 μmol of $MgCl_2$ or $MnCl_2$, 1.2 μmol of theophylline, 0.6

unit of creatine kinase, 37.5 nmol of GTP and 0.5 to 1.0 μCi of [8-^{3}H]GTP. The incubations were carried out at 37° for 10 min, and the radioactive cGMP produced was isolated by a procedure described by Deguchi *et al.* [14].

Protein measurement. Protein was determined by the method of Lowry *et al.* [41] using bovine serum albumin as standard.

Preparation of the solutions of the derivatives. Each derivative was initially dissolved in DMSO to make a 10 mM solution and then was diluted to 3.3 mM with water. The diluted solution was added to the assay medium at a final concentration of 0.22 mM.

RESULTS

The effects of twenty-nine derivatives of Arg on crude guanylate cyclase were examined in the presence of Mn^{2+} or Mg^{2+} as a metal cofactor for the enzyme. The guanylate cyclase activity in the 105,000 *g* supernatant fraction of neuroblastoma was not changed significantly by the individual addition of all the derivatives in the presence of Mn^{2+} (Table 1). However, eight derivatives (compounds XIV, XV, XVI, XX, XXII, XXIII, XXIV and XXV) containing *N*α-DNS residue suppressed the activity to 1/6–1/2 of the original level in the presence of Mg^{2+} (Table 1). The *N*α-DNS derivatives of Arg further modified at the guanidino group (position R_1) with a 2-imino-1,2,3,4,5,6-hexahydropyrimidin-4-yl (IHHP-4-yl) or 2-imino-1,2,3,4,5,6-hexahydropyrimidin-5-yl (IHHP-5-yl) group still exhibited the inhibitory effect on the enzyme. All the *N*α-benzoyl-, *N*α-tosyl- and *N*α-naphthyl-Arg derivatives tested were ineffective. The inhibitory action could not be ascribed to the effect of DMSO, the solvent in which these derivatives were dissolved, since DMSO did not affect significantly the enzyme activity. Sodium nitroprusside, a potent activator of soluble guanylate cyclase [18], had no effect, since the supernatant fraction contained an activated enzyme as suggested by Deguchi and Yoshioka [38] (data not shown). As shown in Fig. 1, compound XX caused a significant change of the affinities of the crude guanylate cyclase against GTP without altering the V_{max} value, indicating that the mode of the inhibition was competitive. The apparent K_m values were 0.33 and 1.43 mM without and with the inhibitor respectively. The K_i values of compounds XIV–XVI, XX and XXII–XXV are summarized in Table 1.

The effects of the Arg derivatives on guanylate cyclase partially purified by the Chelex 100 Na^+ column, which adsorbs almost all the cations in the 105,000 *g* supernatant fraction, were examined further in the presence of Mg^{2+}. The activity of partially purified guanylate cyclase decreased to 10–20% of the original found in the supernatant fraction. As shown in Fig. 2, the reduction in activity was reversed to a range of 40–80%, (depending on the protein content) by the addition of Arg (I) and Arg ME (VII) as reported by Deguchi and Yoshioka [38]. Among the derivatives, M Arg ME (X) produced up to 80% activation of the Arg-evoked guanylate cyclase activity. The K_A values of Arg (I), Arg ME (VII) and M Arg ME (X) were 3.2, 4.4 and 9.2 μM respectively. These active principles were

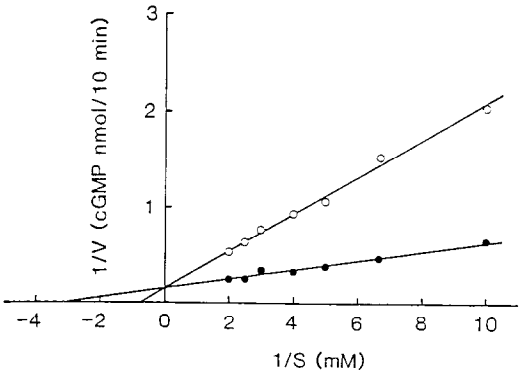


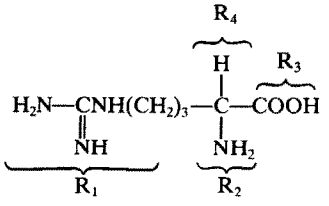
Fig. 1. Competitive inhibition of compound XX with crude guanylate cyclase against GTP concentration in the presence of $MgCl_2$. The supernatant fraction (60 μg protein) of neuroblastoma cells was used for each assay. The activity was measured in the presence (—○—) or absence (—●—) of 0.044 mM compound XX.

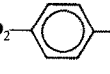
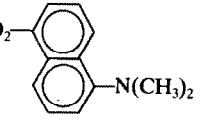
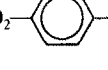
inactivated by removal of —COOH as shown in agmatine (II), amidation of —COOH in Argamide (VI), modification of the guanidino group with a tosyl group in N^g-tosyl Arg (XVII), cyclarization of the side chain (R_1) of Arg in 2-amino-2-(IHHP-4-yl)acetic acid (XVIII) and 2-amino-3-(IHHP-5-yl)propionic acid (XXI), and modification of —NH_2 with a *N*α-benzoyl, *N*α-tosyl, *N*α-naphthyl or *N*α-DNS group. Compounds V, XIV, XV, XVI, XX, XXII, XXIII, XXIV and XXV also suppressed the basal activity to about 1/6 to 1/3 (Fig. 2). As shown in Fig. 3, compound XX competitively inhibited the basal activity but noncompetitively inhibited the Arg-evoked guanylate cyclase activity. Arg did not change the K_m value of 3.42 mM of the Chelex-passed guanylate cyclase against GTP, but increased the V_{max} value. The K_i values of compounds V, XIV, XV, XVI, XX, XXII, XXIII, XXIV and XXV were 28, 16, 2.5, 13, 3.1, 9.9, 12, 8.5 and 8.1 μM respectively. The inhibitory effects of compounds XV and XX were the strongest among these nine inhibitors.

DISCUSSION

In the present studies, we found that a new synthesized compound, M Arg ME (X), as well as Arg (I) and Arg ME (VII), could activate the Chelex-passed guanylate cyclase. The free guanidino and —NH_2 residue of Arg were essential for the activation since the active principles lost the activating potencies after modification of the guanidino and/or —NH_2 residue. In addition, neither agmatine (II) nor Argamide (VI) activated the activity, suggesting that the —COO— group is also important in the activation. On the other hand, among the Arg derivatives, eight compounds which contain a free guanidino group including IHHP-4-yl or IHHP-5-yl and a *N*α-DNS residue in their molecules exerted a prominent suppressive effect on the soluble guanylate cyclase. Although DNS-Arg (V) was not an inhibitor and compound XIV was a weak inhibitor, introduction of a hydrophobic ring such as cyclohexane into compound XVI made compound XIV a

Table 1. Structures of Arg derivatives, and their effects on soluble guanylate cyclase



	R_1	R_2	R_3	R_4	cGMP (nmol/10 min) $MnCl_2$	cGMP (nmol/10 min) $MgCl_2$	K_i (μM)
I	$H_2N—C(=NH)NH(CH_2)_3—$	NH_2	COOH	H	1.16	0.92	
II	〃	NH_2	H	H	1.07	0.95	
II	〃	NHCO– 	COOH	H	1.03	0.89	
IV	〃	$NHSO_2$–  –CH_3	COOH	H	1.09	0.96	
V	〃		COOH	H	1.17	0.85	
VI	〃	NH_2	$CONH_2$	H	1.10	0.96	
VII	〃	NH_2	$COOCH_3$	H	1.05	0.93	
VIII	〃	NHCO– 	$COOCH_3$	H	1.03	0.90	
IX	〃	$NHSO_2$–  –CH_3	$COOCH_3$	H	1.10	0.92	
X	〃	NH_2	$COOCH_3$	CH_3	1.10	1.01	
XI	〃	$NHSO_2$–  –CH_3	$COOCH_3$	CH_3	1.22	0.96	

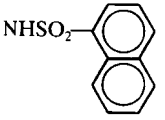
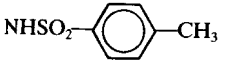
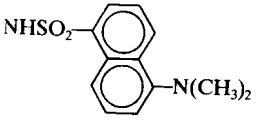
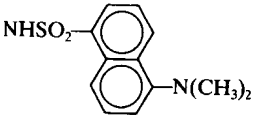
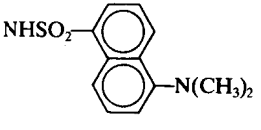
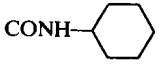
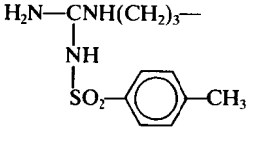
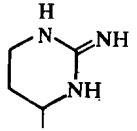
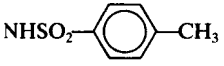
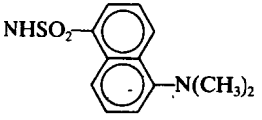
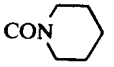
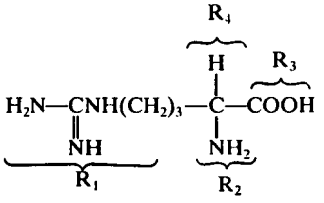
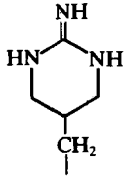
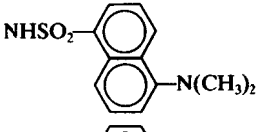
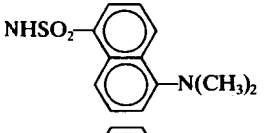
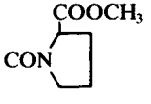
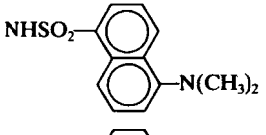
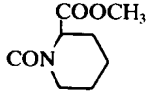
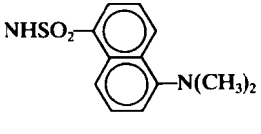
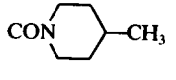
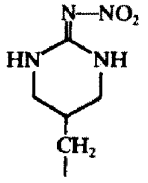
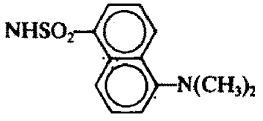
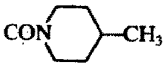
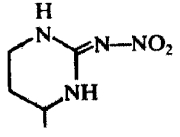
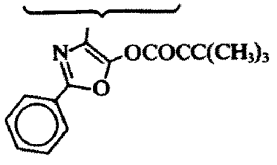
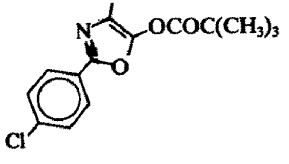
XII	〃		$COOCH_3$	CH_3	1.13	0.96	
XIII	〃		COOH	CH_3	1.25	0.98	
XIV	〃		COOH	CH_3	1.20	0.45	6.3
XV	〃		$COOC_3H_7$	CH_3	1.10	0.25	1.9
XVI	〃			CH_3	1.20	0.11	1.7
XVII		NH_2	COOH	H	1.09	0.92	
XVIII		NH_2	COOH	H	1.03	0.88	
XIX	〃		$COOC_3H_7$	H	1.00	0.98	
XX	〃			H	1.06	0.14	1.8

Table 1. Continued



	R_1	R_2	R_3	R_4	cGMP (nmol/10 min) $MnCl_2$	cGMP (nmol/10 min) $MgCl_2$	K_i (μM)
XXI		NH_2	COOH	H	1.18	0.99	
XXII	〃		$COOC_4H_9$	H	1.18	0.21	7.5
XXIII	〃			H	1.21	0.26	2.9
XXIV	〃			H	1.18	0.23	6.2
XXV	〃			H	1.17	0.15	2.7

XXVI				H	1.26	0.93
XXVII					1.15	0.83
XXVIII	〃				1.13	0.84
XXIX	Acetyl-Leu-Leu-Arginal (Leupeptine)	—	CHO	H	1.10	0.88
None*	(control)				1.08	0.89
DMSO†					1.05	0.91

The supernatant fraction (58 μg protein) of neuroblastoma cells was used for each assay. Results are shown as the means (N = 3).
* Basal guanylate cyclase activity in the absence of Arg derivatives.
† Basal guanylate cyclase activity obtained by adding DMSO to the assay medium in place of Arg derivatives.

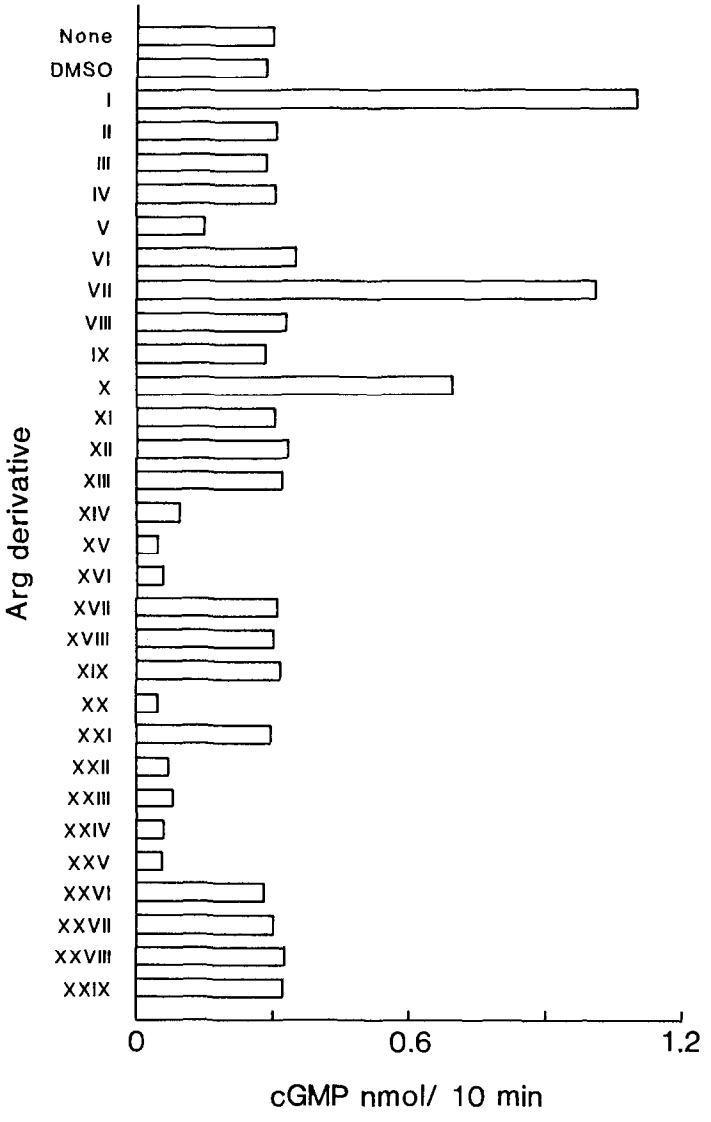


Fig. 2. Effects of Arg derivatives on the Chelex-passed guanylate cyclase in the presence of $MgCl_2$. The Chelex 100 Na^+ effluent (40 μg protein) was used for each assay. Guanylate cyclase activity was measured in the presence of each Arg derivative (0.22 mM). Results are shown as the means (N = 3).

stronger inhibitor. Therefore, blocking the carboxyl residue seemed to be required for expressing the inhibitory activity. Although Arg is not a substrate for the guanylate cyclase, the kinetic studies suggested that compound XX apparently inhibited the enzyme competitively due to the competition with Arg on the enzyme. A structure of compound XX, a representative inhibitor, is shown in Fig. 4.

Sherry *et al.* [42] showed that TAME (IX) was not only a substrate of thrombin, which possesses rigid substrate specificity on -Arg-Gly- residues within fibrinogen, but also a weak inhibitor of the enzyme. In the development of a series of thrombin inhibitors, Okamoto *et al.* [43] clearly demonstrated that modification of $—NH_2$ of Arg ME with acetyl, $N\alpha$-tosyl, $N\alpha$-naphthyl and $N\alpha$-DNS groups, in turn, made the inhibition stronger, and confirmed that the aromatically hydrophobic interaction of the inhibitor with thrombin was one of the inhibitory factors. The soluble guanylate cyclase from neuroblastoma was inhibited by DNS-Arg derivatives but not by $N\alpha$-benzoyl-, $N\alpha$-tosyl- and $N\alpha$-naphthyl-Arg derivatives. These results suggested that the dimethylamino group is involved in the inhibition. As compounds XX and XXV have a common structure which is modified with a DNS group and piperidine except for the difference of a side chain (R_1) of Arg, the K_i values for the crude guanylate cyclase are nearly the same. Although structural differences between compounds XXIV and XXV are 2-carboxylic acid methyl ester and 4-methyl, the K_i value of compound XXIV for the crude guanylate cyclase was about 2-fold larger than that of compound XXV. Kikumoto *et al.* [44] synthesized strong thrombin inhibitors from Arg derivatives, in which $—NH_2$ and —COOH were modified with a quinoline and piperidine group respectively. In the inhibitors, there existed four stereoisomers due to the piperidine moiety which

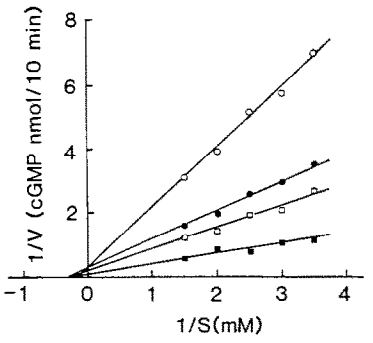


Fig. 3. Double-reciprocal plots of the Chelex-passed guanylate cyclase activity against GTP concentration in the presence of Arg and compound XX. The Chelex 100 Na^+ effluent (45 μg protein) was used for each assay. Guanylate cyclase activity was measured in the absence (—●—) or presence (—○—) of 0.02 mM compound XX, the presence (—■—) of 0.02 mM Arg, and the co-existence (—□—) of 0.02 mM Arg and 0.02 mM compound XX.

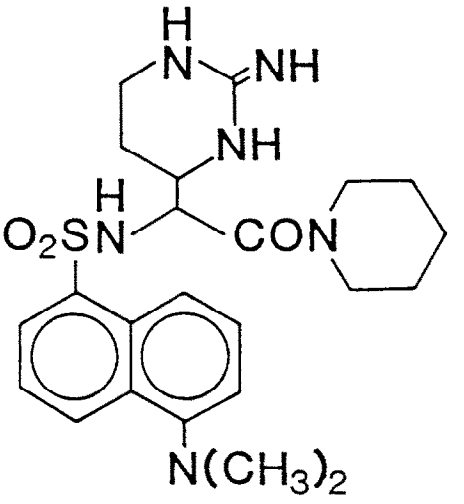


Fig. 4. Structure of compound XX which possesses an inhibitory effect on guanylate cyclase.

were substituted at positions 2 and 4 with carboxylic acid and a methyl group respectively. They found that the strongest inhibitor was 15,000-fold more effective than the weakest one. These results suggest that there is a possibility of creating a stronger inhibitor for guanylate cyclase by the introduction of the piperidine moiety into the Arg derivatives.

Among eight inhibitors, the compounds composed of a guanidino group or IHHP-4-yl as the side chain (R_1) of Arg were relatively stronger inhibitors than the compounds possessing a IHHP-5-yl group. Taking the activation of guanylate cyclase by not only Arg but also homoarginine [38] into consideration, the stronger inhibitors might be established by the modification of a side chain such as the change of the length from a Cα-carbon to a guanidino group and changes of the structures of the guanidino group relating to the side chain.

The intracellular cGMP content in the neuroblastoma N1E 115 cells and the influx of calcium into the cells were increased markedly by adding phosphatidic acid to the incubation medium [6]. One of the mechanisms of cGMP augmentation in the cells may be due to activation of the soluble guanylate cyclase by the interaction of Arg, calcium ion and endogenous essential factors. The contribution of Arg to guanylate cyclase activation will be confirmed, if the Arg derivatives possessing the inhibitory effect on the enzyme suppress the cGMP augmentation and calcium influx into the cells.

Endothelium-derived relaxing factor has been known to play an important role not only on the relaxation of vascular tissue [33] but also on the inhibition of platelet aggregation [45, 46] and platelet adhesion [47]. Recently, the factor seems to be identified as nitric oxide which is formed from the guanidino nitrogen atom of L-Arg by an unestablished mechanism in vascular endothelial cells. Nitric oxide release in the cells was enhanced by L-citrulline but not by ornithine, L-α-amino γ-guanidino butyric acid, homoarginine, guanidine hydrochloride, canavanine and L-arginyl-aspartate. In contrast, the activity of soluble guanylate cyclase in the neuroblastoma was enhanced by homoarginine and L-arginyl-aspartate but not citrulline [38]. The response to Arg-relating compounds is different between release of nitric oxide from the endothelial cells and activation of the guanylate cyclase. This result suggests that nitric oxide is not involved in the mechanism of activation of guanylate cyclase by Arg.

Hibbs *et al.* [48] have demonstrated that L-Arg or homoarginine is required for the expression of the activated macrophage cytotoxic factor but D-Arg, agmatine or TAME is not. Deimination and oxidation are believed to be coupled in this mechanism [49]. They also pointed out that N^g-monomethyl-L-arginine is a competitive inhibitor of the activated macrophage. The response to Arg derivatives is similar to that for the activation of the guanylate cyclase. However, the mechanism may be different because endogenous essential factors (data to be published) are required for the activation of the guanylate cyclase by Arg. Of course, it is of interest to know whether or not our synthesized Arg derivatives are capable of affecting the release of nitric oxide and the macrophage cytotoxicity. In addition, the effects of canavanine and N^g-methyl-L-arginine on soluble guanylate cyclase should be evaluated.

Thus, the inhibitor and activator found in this study may shed some insight concerning the elucidation of the activation mechanism of guanylate cyclase by Arg and the participation of guanylate cyclase in the neuronal transmission.

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