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Identification and Characterization of Endothelin Receptors in Rat Cerebellum

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ABSTRACT

Endothelins (ETs), potent vasoconstrictors, are also neuropeptides in mammalian brain. The objectives of this study were to identify and characterize the receptors for ETs in rat cerebellum, using radioligand binding techniques and affinity cross-linking method. Iodinated ET-1 bound specifically to receptors in rat cerebellar membrane in a dose-dependent manner. Scatchard plot indicated a single class of high-affinity binding sites. Apparent K_d value was 110 pM, and the B_{max} value was 4.85 pmol/mg of membrane protein. Through experiments of affinity cross-linking of ET receptors with ^{125}I-ET-1, two forms of endothelin receptors with molecular masses of 47 KD and 32 KD were identified. Electrophoresis conducted in the presence of reducing reagents did not affect the mobilities of specifically labeled bands, suggesting that endothelin receptors exist as a single polypeptide chain. The order of potency for ETs and sarafotoxin 6b (S6b) in displacing the specifically bound iodinated ET-1 from cerebellar membrane was ET-1 = ET-2 = ET-3 > S6b. Result from receptor specificity indicated that endothelin receptors in rat cerebellum belong to endothelin receptor subtype B.

Key Words: Endothelin receptor, Rat cerebellum, Radioligand binding techniques, Affinity cross-linking method.

INTRODUCTION

Endothelins (ETs) are a group of peptides originally discovered from cultured porcine aortic endothelial cells [1]. Genomic analysis indicates there are three isoforms of endothelin: ET-1, ET-2, and ET-3. Each isoform has more than 70% amino acid sequence homology to the others [2]. These isoforms encoded by separate genes and all possess potent vasoconstrictor activity. The mechanism through which endothelins elicit vasoconstriction may involve phospholipase C [3], calcium mobilization either from internal stores or from outside of the cell [3], and protein kinase C[4]. It has been suggested that the physiological responses are initiated by the coupling of G-protein to endothelin receptors. Among the endothelins, ET-1 is the most potent mammalian vasoconstrictor peptide known to date[1]. It has been suggested that ET-1 may play an important role in regulating systemic blood pressure and perhaps local blood flow, and that the disturbance of this regulatory mechanism

could contribute to pathological states of hypertension [5] or vascular spasm.

Besides its potent vasoconstrictor activity, ET-1 also has demonstrated a wide variety of pharmacological effects, including stimulation of the release of eicosanoids (vasodepressors) and endothelium-derived relaxing factor (EDRF) from a perfused vascular bed [6], and those effects involved in the physiology of cardiac [7,8], renal [9], and pulmonary function [10] as well as mitogenesis and tissue remodeling [11,12] . The wide spectrum of pharmacological effects of ET-1 suggests the existence of subtypes of ET-1 receptor. Recently, two cDNAs encoding structurally and functionally distinct endothelin receptors were cloned[13,14]. The order of potency of ET_A receptor is ET-1>ET-2> ET-3, while ET_B receptor displays similar affinities toward each endothelin isoform (i.e., ET-1 = ET-2 = ET-3). Each receptor contains seven transmembrane domains and exhibits significant sequence and topographical similarity with known G-protein-coupled receptors, suggesting that these two endothelin receptors belong to the superfamily of G-protein-coupled receptors.

Several studies have indicated that endothelins also act as neuropeptides in mammalian brain. Both ET-1 and ET-3 have been identified in porcine brain homogenate and in porcine spinal cord [15,16]. In addition, an *in situ* hybridization study[17] revealed widespread distribution of endothelin mRNA in the cerebellum and other brain regions, suggesting that ET in brain may play a fundamental role in regulating nervous system function. Therefore, in this study, we attempted to identify and characterize endothelin receptors in rat cerebellum by a radioligand binding assay and by affinity cross-linking of ET receptors with ^{125}I-ET-1. The identification of abundant high-affinity endothelin receptors present in cerebellar membrane supports the hypothesis that endothelins play not only a vasoregulator role but also a neuromodulator role in the brain.

MATERIALS AND METHODS

I. *Preparation of membranes*.

Cerebellar membranes were prepared from adult male Sprague-Dawley rats. After decapitation, cerebellum were removed, and homogenized by polytron (PT 3000, Kinematica, Switzerland) for 1 min in 10 volumes (v/w) of ice-cold homogenizing buffer, which contained 10 mM Tris-HCl, pH 7.7; 0.32 M sucrose; 5 mM EDTA; 0.1 mM PMSF; 10 μg/ml leupeptin; and 50 μg/ml soybean trypsin inhibitor. All subsequent steps were conducted at 4℃. The homogenate was then centrifuged at 900 x g for 10 min (JA-20, J2-21 M/E, Beckman) and the supernatant (S1) and pellet (P1) were collected separately. The supernatant (S1) was further centrifuged at 46,000 x g for 30 min. After centrifugation, pellets were resuspened in 10 volumes of hypotonic buffer containing 5 mM Tris-HCl, pH 7.7; 50 μg/ml soybean trypsin inhibitor, and 10 μg/ml leupeptin. The suspension sat on ice for 1 hr, and then was repolytroned and recentrifuged at 46,000 x g for 30 min. The resultant pellets containing cerebellar neuronal membrane particulates were stored at -20℃ until use.

II. *Binding assay*.

A binding assay mixture (0.5 ml) containing 10 mM Hepes, pH 7.5; 5 mM $MgCl_2$; 3 mM EDTA; 1 mM EGTA; 10 μg/ml leupeptin; 50 μg/ml soybean trypsin inhibitor, 20 μg/ml bacitracin; 0.1 % bovine serum albumin; and membrane protein (20-30 μg/ml) was incubated with 25-50 pM ^{125}I-ET-1 for 2 hr at 25℃. Nonspecific binding was determined in the presence of 100 nM nonradioactive ET-1. The receptor-^{125}I-ET-1 complex was separated from free ^{125}I-ET-1 by filtration through a Whatman GF/C glass filter [18]. The filters were washed twice with 10 ml cold buffer. The radioactivity of the filter was counted with a γ-counter. Usually, nonspecific binding was about 5-10% of the total binding.

III. *Cross-linking of ET receptor with* ^{125}

Membranes (250 μg of protein/ml, final con-

centration) were incubated in 1.5 ml of binding buffer at 25℃ for 2 hr with 400 pM ^{125} I-ET-1 in the presence or absence of 100 nM unlabeled ET-1. After removal of free radioligands by centrifugation, the resulting pellet was resuspended in 0.4 ml of ice-cold 10 mM Hepes, pH 7.5. Two cross-linking reagents, DST and BS^3 (from Pierce), were used to cross-link ET receptor and ^{125} I-ET-1. Freshly prepared stock solution (50 mM) of the cross-linking reagent (BS^3 dissolved in H_2O, DST dissolved in dimethyl sulfoxide (DMSO)) was added to the suspension to make a final concentration of 0.5 or 1.0 mM. Cross-linking was carried out at 25℃ for 20 min and quenched by the addition of 7.5 μl of 4 M ammonium acetate [19]. Five-fold concentrated SDS-gel sampling buffer (0.313 M Tris-HCl, pH 6.8; 10 % (w/v) SDS; and 40 % (v/v) glycerol) in the presence or absence of 100 mM dithiothreitol was added to the reaction mixture. The samples were boiled for 5 min and then electrophoresed on a 10 % gel by the method of Laemmli [20]. Autoradiography was performed by exposing the destained, dried gel to Kodak XAR-5 film with an intensifying screen at -70℃ for 1-2 weeks.

IV. *Materials*

^{125} I-Endothelin-1 (250 μCi) was purchased from Du Pont-New England Nuclear. Ethyleneglycol bis (sulfosuccinimidylsuccinate) (EGS), disuccinmidyl tartarate (DST), and *bis* (sulfosuccinimidyl) suberate (BS^3) were purchased from Pierce Chemical Co. Endothelin-1, -2, -3 and sarafotoxin 6b (S6b) were supplied by Peninsula Laboratories, INC (Belmont, CA). Sucrose, ethylenediaminetetraacetate (EDTA), phenylmethylsulfonyl fluoride (PMSF), ethylenedioxybis (ethylenenitrilo) tetraacetic acid (EGTA), dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (SDS) were from E. Merck. Bacitracin, leupeptin, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), soybean trypsin inhibitor, dithiothreitol (DTT), and all other reagents were purchased from Sigma Chemical Co.

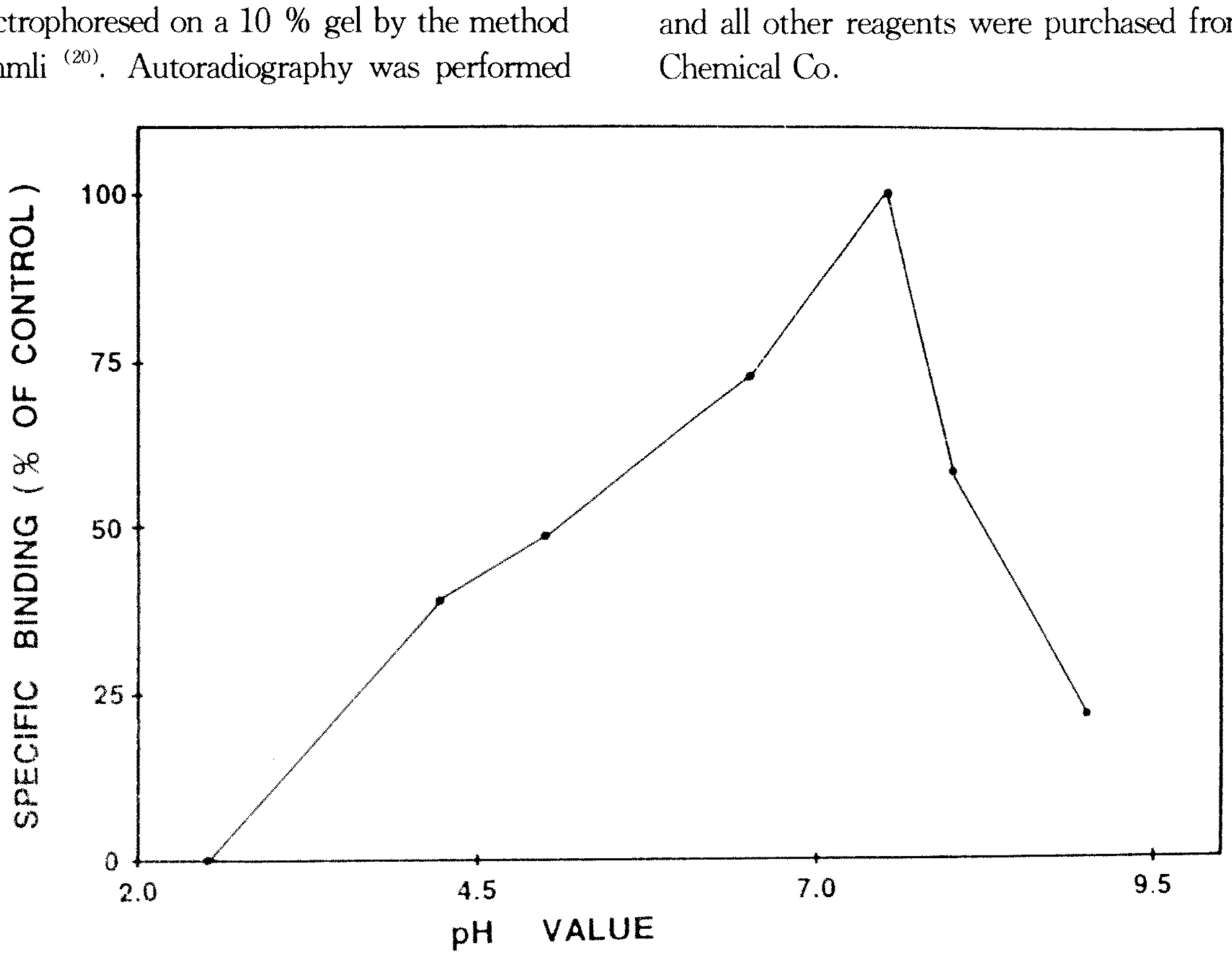

Figure 1. Effect of pH on Specific Binding of ^{125} I-ET-1 to Rat Cerebellar Membranes. About 20 μg/ml of membrane particulates were incubated with 25 pM of ^{125} I-ET-1 at various pH values from 2.5 to 9.0. Each point represents the mean of triplicate values. Control (100%) was arbitrarily assigned at pH 7.5.

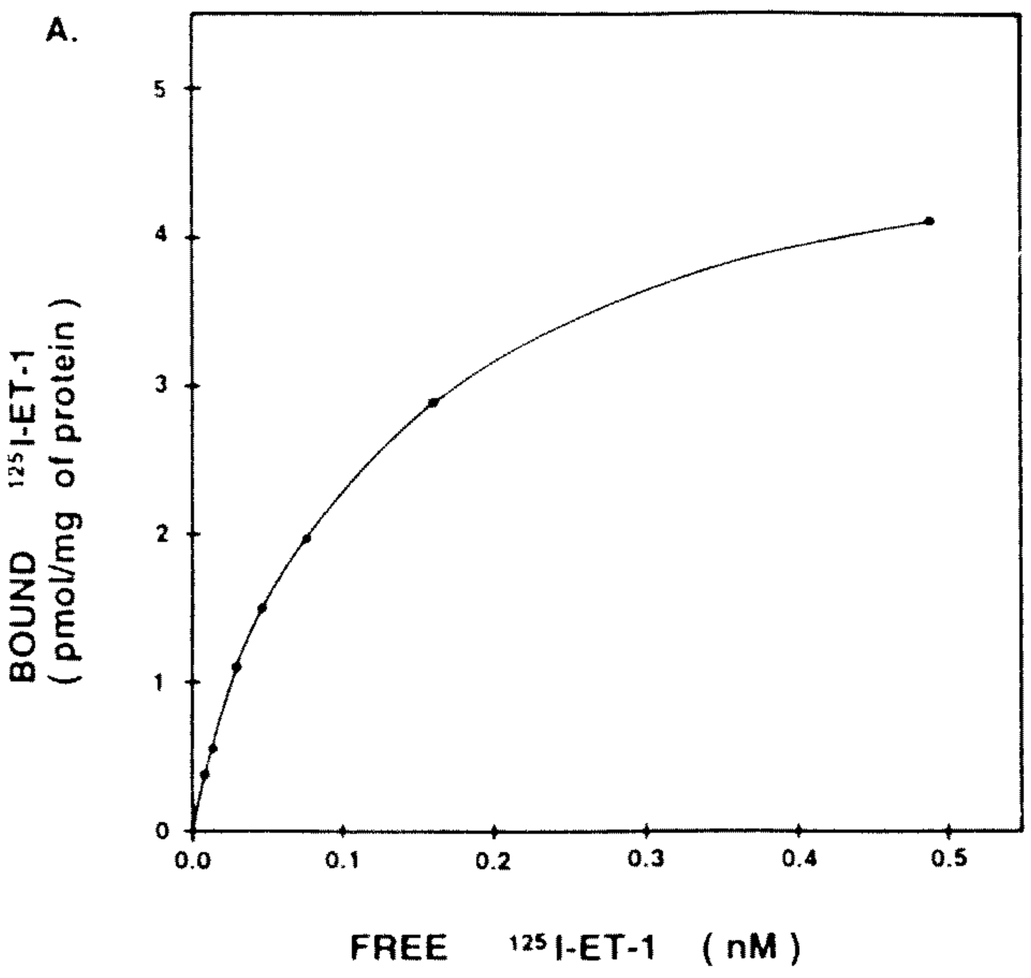

Figure 2. Representative Saturation Curve (A) and Scatchard Plot (B) of Specific ^{125} I-ET-1 Binding Rat Cerebellar Membranes. Membranes were incubated to equilibrium with increasing concentrations of ^{125} I-ET-1 in the absence and presence of 1 μM unlabeled ET-1. Each point represents the mean of duplicate values.

RESULTS

pH Effect

The ^{125} I-ET-1 bound specifically to receptors in rat cerebellar membrane particulates. The effect of pH on specific binding of ^{125} I-ET-1 to cerebellar membranes was examined. As shown in Fig. 1, specific binding was maximal at pH 7.5. Binding was markedly inhibited when pH was above 8.0 or below 5.0.

Saturation Binding Curves

The binding of ^{125} I-ET-1 to cerebellar membrane particulates was saturable (Fig. 2A). Scatchard analysis revealed a single class of high-affinity binding sites (Fig. 2B). The apparent equilibrium dissociation constant (K_d), determined from Scatchard plots, was 110 pM, and the B_{max} value was 4.85 pmol/mg of protein.

Cross-linking of endothelin receptors

^{125}I-endothelin-1 was covalently cross-linked to endothelin receptors in rat cerebellar membrane by the bifunctional reagents disuccinimidyl tartrate (DST) and bis (sulfosuccinimidyl) suberate (BS^3). Autoradiographic analysis of ^{125}I-ET-1-labeled membrane proteins on SDS-PAGE (SDS-polyacrylamide gel electrophoresis) indicated that two labeled bands corresponding to the molecular masses of 50 KD and 35 KD were identified in the membrane preparation from cerebellum (Fig. 3). In addition, these two bands disappeared when the reaction was conducted in the presence of excess unlabeled ET-1. Judging from the density of the labeled bands, a better cross-linking effect was obtained at 1 mM of the bifunctional reagent than at 0.5 mM; also, BS^3 was more efficient than DST in cross-linking ^{125} I-ET-1 to ET receptors.

Subtracting the molecular mass of ^{125} I-ET-1 and that of the cross-linking reagent, the binding protein appeared to be 47 KD and 32 KD. Electrophoresis conducted in the presence of reducing reagent (dithiothreitol, DTT) did not affect the mobilities of specifically labeled bands in cerebellum (data not shown), indicating that endothelin receptors exist as a single type of subunit.

Competition Binding.

To further examine the selectivity of endothelin receptors, ET-1, -2, -3 and sarafotoxin 6b

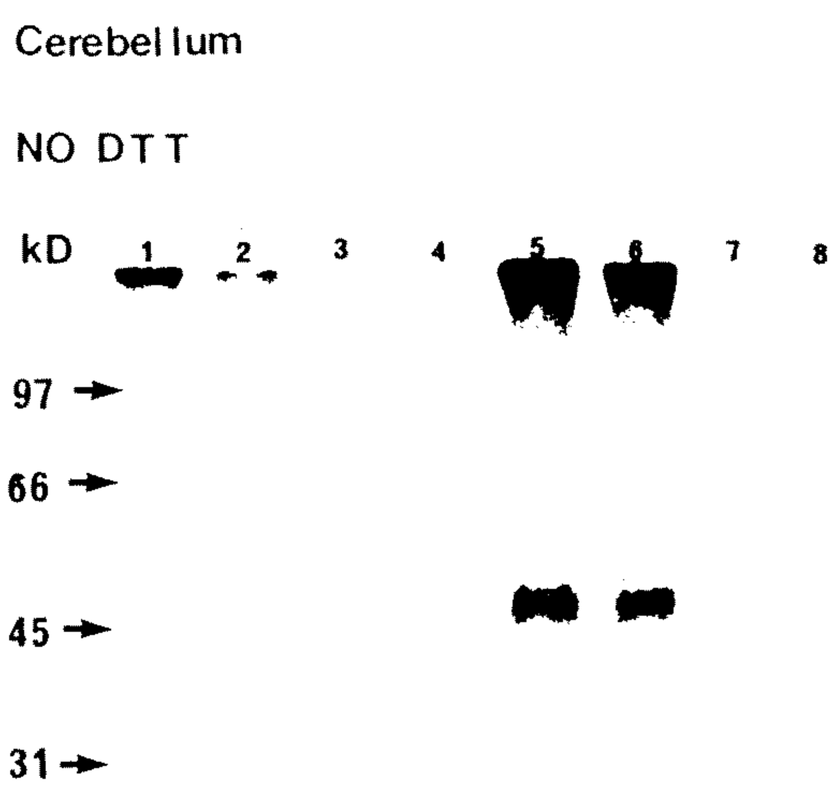

Figure 3. Autoradiogram of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of ^{125} I-ET-1 Covalently Linked to ET Receptors on Rat Cerebellar Membranes. The affinity cross-linking reaction was either conducted in the absence (lanes 1, 2, 5 and 6) or presence of (lanes 3-4, 7-8) 0.1 μM unlabeled ET-1. The cross-linking reagent was DST (lanes 1-4) or BS^3 (lanes 5-8) at 1.0 mM (lanes 1, 3, and 5) or 0.5 mM (lanes 2, 4, and 6).

(S6b) were used as competitors with ^{125} I-ET-1 for binding to cerebellar neuronal membranes. S6b is useful in a competition experiment because it exhibits a remarkable homology of structure as well as function to the ET family [21]. As shown in Fig. 4, ^{125} I-ET-1 was competitively displaced from cerebellar membranes by increasing concentrations of the unlabeled peptides. There was no significantly difference between the competition by ET-1, ET-2, and ET-3, while the competition ability of S6b was significantly lower than that of ET-1, ET-2, and ET-3 (P value < 0.05, t-test). Therefore, inhibiting of ^{125} I-ET-1 binding showed an order of potency of ET-1 = ET-2 = ET-3 > S6b.

DISCUSSION

Results from a radioligand binding assay demonstrated that the binding of ^{125} I-ET-1 to rat cerebellar membranes exhibited the typical receptors kinetics of specificity and saturability. The optimal pH for ^{125} I-ET-1 specific binding was 7.5, which is near physiological condition. In addition, Scatchard analysis of saturation binding indicated a single class of high-affinity binding sites in rat cerebellum. The apparent equilibrium dissociation constant (K_d) of ^{125}I-ET-1 was 110 pM, which is similar to those described for rat aorta [22], rat kidney [23], rat heart [21], etc. The identification of abundant high-affinity endothelin receptors in cerebellar neurons provides supporting evidence for endothelin in brain playing a role in regulating the central nervous system in addition to its role in vasoregulation. Although it was reported that endothelin could stimulate the release of preloaded aspartate from cultured cerebellar granule cells [24], the real physiological role of endothelin in cerebellum remains to be clarified in the future.

The molecular masses of endothelin receptors were further determined by affinity cross-linking of endothelin receptors with ^{125} I-ET-1. Autoradiographic analysis of SDS-PAGE indicated two labeled bands corresponding to 50 KD and 35 KD, which could be blocked by excess unlabeled ET-1, in cerebellum with either the bifunctional reagent DST or BS^3. In addition, similar cross-linking results were obtained without removing the unbound ligand by centrifugation prior to initiating the cross-linking reaction (not shown). The net molecular masses of these two binding proteins were estimated to be 47 KD and 32 KD. Similar molecular masses of binding proteins were reported from several other tissues including chick heart [19], human placenta [25], rat mesangial cells [26], and bovine cerebellum [27], although those from A10 and C6 cell lines were reported to be larger than 60 KD using ethylene glycol bis(succinimidyl succinate) (EGS) as cross-linker [28]. To investigate the possibility that different forms of binding protein might be detected with different cross-linking reagents, a separate experiment with EGS as the bifunctional reagent was also conducted. The cross-linking results did not differ from those obtained with DST or BS^3 (data not shown). Whether or not the difference in molecular mass was caused by the different degrees of receptor gly-

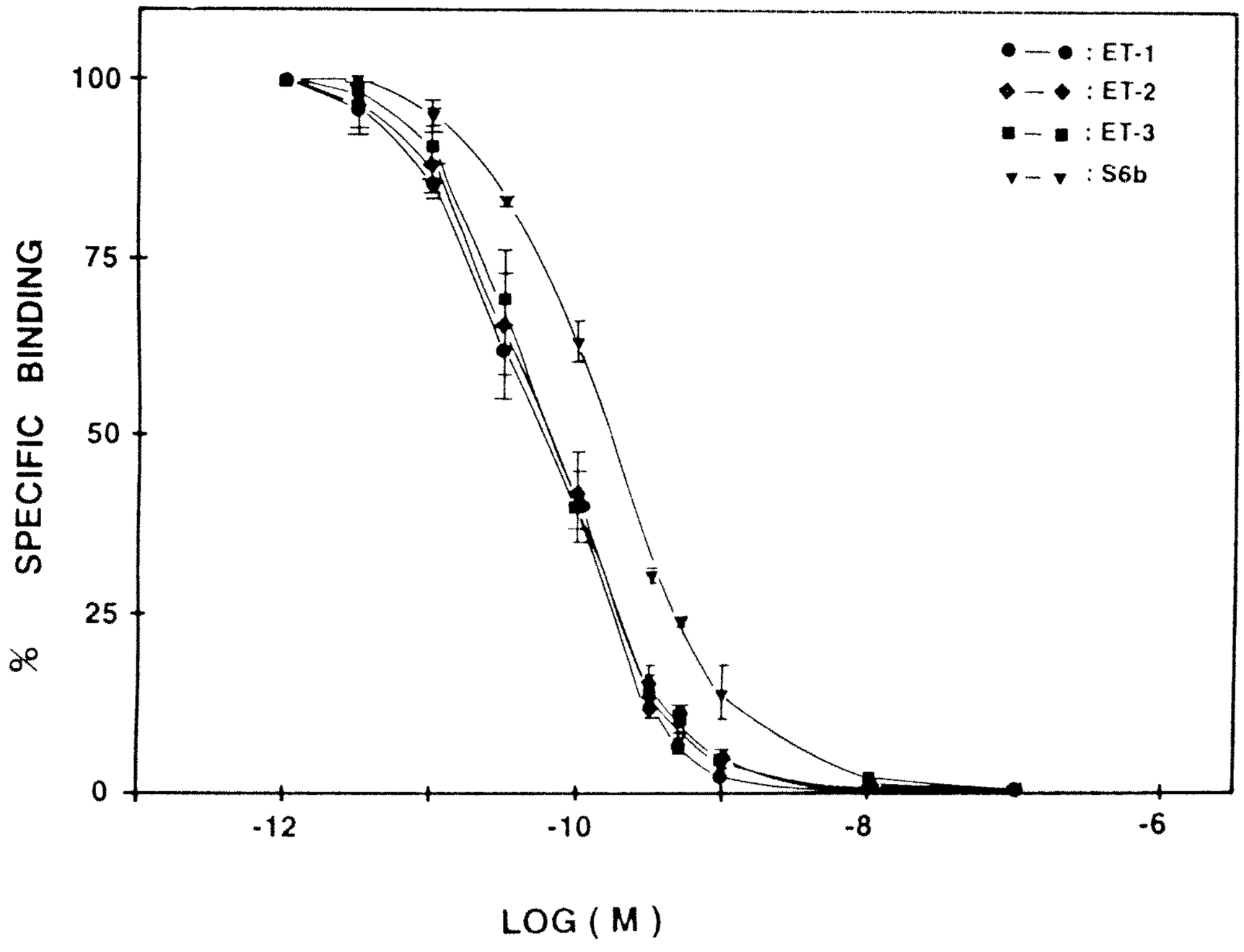

Figure 4. Competition Between ^{125} I-ET-1 and Increasing Concentrations of ET-1, ET-2, ET-3, and Sarafotoxin S6b (S6b). About 20 μg/ml of rat brain cerebellar particulate membrenes wese incubated with fixed concentrations of ^{125}I－ET－1 (25 pM) with the designated concentrations of ET-1, ET-2, ET-3, and S6b at 25℃ for 2 hours. Binding data were corrected for nonspecific binding, which was determined in the parallel incubation in the presence of 0.1 μM and expressed as percentage of the maximal specific ^{125} I-ET-1 binding. Each point represents the mean of two or three separate experiments, each done in triplicate.

cosylation is unclear. It is possible that the smaller (32 KD) form of binding protein was derived from the larger binding protein through proteolytic reaction [27]. Although we used several protease inhibitors in every step, it was inevitable that some proteolysis occurred. In addition, cross-linking experiments performed at 4℃ (not shown) yielded results identical with those observed at 25℃. It is important to note that this proteolysis seemed to have no effect on the affinity of endothelin receptors, since only one receptor affinity was observed in the membrane preparations.

As mentioned earlier, two subtypes of endothelin receptor were cloned recently [13,14]. One, ET_A, shows different affinities for the endothelins ET-1 > ET-2 > ET-3, while the other, ET_B, displays the same affinity toward ET-1, ET-2, and ET-3. Our selectivity studies demonstrated that endothelin receptors in rat cerebellum possessed the similar affinity toward endothelin isoforms, suggesting they belong to an ET_B-like subtype endothelin receptor.

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REFERENCES

1. Yanagisawa, M., Kurihara, H., Kimura, S.,

Tomobe, Y. Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., and Masaki, T. 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411-415.

2. Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., and Masaki, T. 1989. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. U.S.A. 86:2863-2867.

3. Marsden, P. A., Danthuluri, N. R., Brenner, B. M., Ballermann, B. J. and Brock, T. A. 1989. Endothelin action on vascular smooth muscle involves inositol trisphosphate and calcium mobilization. Biochem. Biophys. Res. Commun. 158:86-93.

4. Sugiura, M., Inagami, T., Hare, G. M. T., and Johns, J. A. 1989. Endothelin action: inhibition by a protein kinase inhibitor and involvement of phosphoinositols. Biochem. Biophys. Res. Commun. 158:170-176.

5. Kohno, M., Yasunari, K., Murakawa, K.-I., Yokokawa, K., Horio, T., Fukui, T., and Takeda, T. 1990. Plasma immunoreactive endothelin in essential hypertension. Am. J. Med. 88:614-618.

6. de Nucci, G., Thomas, R., D' Orleans-Juste, P., Antunes, E., Walder, C., Warner, T. D., and Vane, J. R. 1988. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. Proc, Natl. Acad. Sci. U. S. A. 85:9797-9800.

7. Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K., and Masaki, T. 1989. Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria. Am. J. Physiol. 255:H970-H973.

8. Fukuda, Y., Hirata, Y,. Yoshimi, H., Kojima, T., Kobayashi, Y., Yanagisawa, M., and Masaki, T. 1988. Endothelin is a potent secretagogue for atrial natriureitc peptide in cultured rat atrial myocytes. Biochem. Biophys. Res. Commun. 155:167-172.

9. Rakugi, H., Nakamaru, M., Saito, H., Higaki, J., and Ogihara, T. 1988. Endothelin inhibits renin release from isolated rat glomeruli. Biochem. Biophys. Res. Commun. 155: 1244-1247.

10. Lagente, V., Chabrier, P. E., Mencia-Huerta, J. -M., and Braquet, P. 1989. Pharmacological modulation of the bronchopulmonary action of the vasoactive peptide, endothelin, administered by aerosol in the guinea-pig. Biochem. Biophys. Res. Commun. 158: 625-632.

11. Komuro, I., Kurihara, H., Sugiyama, T., Takaku, F., and Yazaki, Y. 1988. Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. FEBS Lett. 238:249-252.

12. Takuwa, N., Takuwa, Y., Yanagisawa, M., Yamashita, K., and Masaki, T. 1989. A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. J. Biol. Chem. 264: 7856-7861.

13. Arai, H., Hori, S., Aramori, I., Ohkubo, H., and Nakanishi, S. 1990. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 348:730-732.

14. Sukurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K., and Masaki, T. 1990. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 348:732-735.

15. Shinmi, O., Kimura, S., Yoshizawa, T., Sawamura, T., Uchiyama, Y., Sugita, Y., Kanazawa, I., Yanagisawa, M., Goto, K., and Masaki, T. 1989. Presence of endothelin-1 in porcine spinal cord: isolation and sequence determination. Biochem. Biophys. Res. Commun. 162:340-346.

16. Shinmi, O., Kimura, S., Sawamura, T.,

Sugita, Y., Yoshizawa, T., Uchiyama, Y., Yanagisawa, M., Goto, K., Masaki, T., and Kanazawa, I. 1989. Endothelin-3 is a novel neuropeptide: isolation and sequence determination of endothelin-1 and endothelin-3 in porcine brain. Biochem. Biophys. Res. Commun. 164:587-593.

17. MacCumber, M. W., Ross, C. A., Glaser, B. M., and Snyder, S. H. 1989. Endothelin: visualization of mRNAs by *in situ* hybridization provides evidence for local action. Proc. Natl. Acad. Sci. U. S. A. 86:7285-7289.

18. Takayanagi, R., Inagami, T., Snajdar, R. M., Imada, T., Tamura, M., and Misono, K. S. 1987. Two distinct forms of receptors for atrial natriuretic factor in bovine adrenocortical cells. J. Biol. Chem. 262:12104-12113.

19. Watanabe, H., Miyazaki, H., Kondoh, M., Masuda, Y., Kimura, S., Yanagisawa, M., Masaki, T., and Murakami, K. 1989. Two distinct types of endothelin receptors are present on chick cardiac membranes. Biochem. Biophys. Res. Commun. 161:1252-1259.

20. Laemmli, U. K. 1970. Cleavage of standard proteins during assembly of the head of bacteriophage T_4. Nature (London)227:680-685.

21. Gu, X. -H., Casley, D. J., and Nayler, W. G. 1989. Sarafotoxin S6b displaces specifically bound ^{125} I-endothelin. Eur. J. Pharmacol. 162:509-510.

22. Hirata, Y., Yoshimi, H., Takata, S., Watanabe, T. X., Kumagai, S., Nakajima, K., and Sakakibara, S. 1988. Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 154:868-875.

23. Jones, C. R., Hiley, C. R., Pelton, J. T., and Miller, R. C. 1989. Autoradiographic localisation of endothelin binding sites in kidney. Eur. J. Pharmacol. 163:379-382.

24. Lin W.-W., Lee. C. Y., and Chuang, D.-M. 1990. Endothelin-1 stimulates the release of preloaded [^{3}H] D-aspartate from cultured cerebellar granule cells. Biochem. Biophys. Res. Commun. 167:593-599.

25. Nakajo, S., Sugiura, M., Snajdar, R. M., Boehm, F. H., and Inagami, T. 1989. Solubilization and identification of human placental endothelin receptor. Biochem. Biophys. Res. Commun. 164:205-211.

26. Sugiura, M., Snajdar, R. M., Schwartzberg, M., Badr, K. F., and Inagami, T. 1989. Identification of two types of specific endothelin receptors in rat mesangial cell. Biochem. Biophys. Res. Commun. 62:1396-1401.

27. Schvartz, I., Ittoop, O., and Hazum, E. 1990. Identification of endothelin receptors by chemical cross-linking. Endocrinology 126: 1829-1833.

28. Martin, E. R., Brenner, B. M., and Ballermann, B. J. 1990. Heterogeneity of cell surface endothelin receptors. J. Biol. Chem. 265:14044-14049.

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鼠小腦中內皮素受體之鑑定及其性質之探討

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摘　要

內皮素(endothelins)不但是種强烈血管收縮劑,也是一種位於哺乳動物腦中的神經胜肽.本研究主要是利用放射性配位體結合技術以及親和性交互聯結作用之方法,來作爲對鼠小腦中內皮素受體之鑑定及其性質之探討.結果發現碘 125 所標識之內皮素-1 可專一性地與鼠小腦上之受體結合,並且呈現量化之關係.由斯可恰圖(Scatchard plot)之結果看出,只有一種具高親和性之接受體存在於鼠小腦中,其解離常數(dissociation constant;Kd)爲 110 pM,而最大之結合位置(maximum binding site;Bmax)爲每毫克之膜蛋白中便有 4.85 pmol 之接受體存在.以碘 125 所標識之內皮素-1 與其受體作親和性交互聯結作用後,經由電泳分析發現有兩種分子量大小之內皮素受體,一爲 47,000,另一爲 32,000,較小分子量之受體可能乃是較大分子量之受體經由水解蛋白之酵素作用而來的.另亦發現此接受體在電泳中之移動速率並不受是否有還原劑存在之影響,這表示內皮素受體乃是以單一多胜鏈之形式存在.進一步地利用競爭性結合實驗,發現鼠小腦之內皮素體對於內皮素及 sarafotoxin 6b (S6b)之親和力爲內皮素-1 = 內皮素-2 = 內皮素-3 >S6b.由本研究之結果,可在鼠小腦中鑑定到相當量且高親和力之內皮素受體之存在,這支持了內皮素具有調節腦神經功能之假說,另由其受體專一性之結果得知鼠小腦中之內皮素受體是屬於 B 亞型之內皮素受體。