36) Specific Binding Sites for Endothelins in the Central Nervous System.
Masami Niwa, Meiko Fujimoto, Yasufumi Kataoka, and Kohtaro Taniyama.
Department of Pharmacology 2, Nagasaki University School of Medicine, Nagasaki
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Endothelins (ETs) seem to play the role of neurotransmitters and/or neuro-modulators in the central nervous system. The receptor autoradiographic method we used previously revealed that specific binding sites of ^{125}I -ET-1, candidates for physiologically active receptors for ETs are heterogeneously distributed in the rat brain. The method also provided evidence of the presence of two distinct binding sites, one a high-affinity site (0.6 nM of Kd) in the choroid plexus and lacunosum molecular layer of the hippocampus, the other a low-affinity site (5 nM) in the subfornical organs and granular layer of the cerebellum. The possible existence of multiple ET receptors in the rat brain led to use of an affinity cross-linking technique with bis (sulfosuccinimidyl) suberate (BS) and ^{125}I -ET-1.

Male 12-week-old Wistar rats were decapitated, and the cerebellum, fore-brain, choroid plexus and lung were immediately removed, immersed in isopentane, and stored at -80°C . Six-month-old pigs were slaughtered, and the thoracic spinal cord was removed within 1 hr. The human thoracic spinal cord was obtained at autopsy on a male adult who died of hepatoma at the age of 65 years. The interval between death and autopsy was 3.5 hr. These spinal cords were immersed in isopentane at -30°C , and stored at -80°C . The tissues were homogenized in 20 volumes of 0.32 M sucrose containing 10 mM EDTA-2Na, 0.1 mM phenylmethylsulphonyl fluoride, 4 $\mu\text{g}/\text{ml}$ leupeptin and 1 mg/ml bacitracin. After centrifugation at $1000 \times g$ for 10 min, the supernatant was recentrifuged at $36000 \times g$ for 20 min. The pellet was suspended in the binding buffer, 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM EDTA-2Na, 100 mM NaCl, 4 $\mu\text{g}/\text{ml}$ leupeptin, 2 $\mu\text{g}/\text{ml}$ chymostatin and 1 mg/ml bacitracin. The membranes were incubated with ^{125}I -ET-1 at 25°C . After incubation for 30 min, BS was added in a final concentration of 0.78 mM, and allowed to react for 25 min. The reaction was quenched and the membranes cross-linked with the radioligands were solubilized at 100°C for 3 min in the sample buffer. After solubilized, the membranes were analyzed by electrophoresis on 5-20% SDS-polyacrylamide gels (PAGE). Autoradiograms were obtained from the dried gels after exposure to Kodak X-Omat AR film with enhancing screen at -80°C . The bands were excised from the dried gels and the radioactivity determined.

Chemical cross-linking technique with BS and membranes obtained from the rat cerebellum, forebrain and lung followed by SDS-PAGE revealed that ^{125}I -ET-1 labeled two bands with molecular weights of 43 kDa and 33 kDa. In the case of the rat choroid plexus, two bands of 51 kDa and 33 kDa were specifically labeled. The human and porcine spinal cords also had two bands of 43 kDa and 33 kDa. The 33 kDa band was of very low-density, when the tissues were homogenized in 0.32 M sucrose containing 10 mM EDTA-2Na, indicating the possibility that 33 kDa band is a degradation product of the larger band. Unlabeled ET-1 inhibited the binding of ^{125}I -ET-1 to the rat cerebellar membranes with a high affinity and the intensity of the labeled band of 43 kDa was reduced in parallel with the decreased binding.

Noteworthy was the difference in the molecular weight of specific ^{125}I -ET-1 binding protein between the rat cerebellum and choroid plexus, a finding which suggests heterogeneity of ETs receptors. Although there is little evidence as to whether ETs function as neurotransmitters or neuromodulators, data recently obtained revealed that ET-1 releases substance P from the newborn rat spinal cord preparation. The present finding of the possible existence of ETs receptors in the spinal cord led to the suggestion that ETs play the role of neurotransmitters and/or neuromodulators in the central nervous system.