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Decreases in rat brain aquaporin-4 expression following intracerebroventricular administration of an endothelin ET_B receptor agonist

Yutaka Koyama*, Kazuhiro Tanaka

Laboratory of Pharmacology, Faculty of Pharmacy, Osaka Ohtani University, 3-11-1 Nishikiori-kita, Tonda-bayashi, Osaka 584-8540, Japan

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ABSTRACT

Aquaporins (AQPs) comprise a family of water channel proteins, some of which are expressed in brain. Expressions of brain AQPs are altered after brain insults, such as ischemia and head trauma. However, little is known about the regulation of brain AQP expression. Endothelins (ETs), vasoconstrictor peptides, regulate several pathophysiolgical responses of damaged nerve tissues via ET_B receptors. To show possible roles of ET_B receptors in the regulation of brain AQP expression, the effects of intracerebroventricular administration of an ET_B agonist were examined in rat brain. In the cerebrum, the copy numbers of AQP4 mRNAs were highest among AQP1, 3, 4, 5 and 9. Continuous administration of 500 pmol/day $Ala^{1,3,11,15}$-ET-1, an ET_B selective agonist, into rat brain for 7 days decreased the level of AQP4 mRNA in the cerebrum, but had no effect on AQP1, 3, 5 and 9 mRNA levels. The level of AQP4 protein in the cerebrum decreased by the administration of $Ala^{1,3,11,15}$-ET-1. Immunohistochemical observations of $Ala^{1,3,11,15}$-ET-1-infused rats showed that GFAP-positive astrocytes, but not neurons, activated microglia or brain capillary endothelial cells, had immunoreactivity for AQP4. These findings indicate that activation of brain ET_B receptors causes a decrease in AQP4 expression, suggesting that ET down-regulates brain AQP4 via ET_B receptors.

Aquaporins (AQPs), which comprise a family of water channel proteins with more than 10 members, promote water movements across the plasma membrane in all tissues. AQP1, 3, 4, 5 and 9 are expressed in nerve tissues and brain cells, and the regulation of their expression has been widely studied [7,18,24,27]. For example, AQP4, a major AQP in brain, is primarily located in astrocytes around brain capillaries [24]. After ischemia in mouse brains, expression of AQP4 in astrocytes is altered in a bi-phasic manner, with a transient decrease followed by a gradual increase [3]. In rat traumatic brain injury models, a decrease in AQP4 expression at the damaged sites was observed [5,6]. The level of brain AQP9, which was observed in a sub-population of neurons and astrocytes, increased after brain ischemia [1]. The significance of these altered AQP expressions is discussed in relation to the pathophysiological responses of damaged brain, especially to brain edema. Brain edema is a mortal pathological state and commonly occurs after brain insults. Studies in AQP-deletion mice showed that some AQPs are responsible for the generation of brain edema and/or its improvement [16,20,21]. Several chemical mediators released in damaged nerve tissues were shown to modulate the expression of brain AQPs [4,13,25,26]. However, the regulatory mechanisms underlying AQP expression after brain insults remain to be clarified.

Production of endothelins (ETs), vasoconstrictor peptides, in brain, is increased by brain ischemia and traumatic nerve injuries [19]. Increased levels of brain ET-1 have aggravating actions on ischemic damage and brain edema via ET_A receptors [2,14,15,17]. The other type of ET receptors, ET_B receptors, is predominantly expressed in brain [22]. Intracerebroventricular administration of an ET_B receptor agonist stimulated phenotypic conversion of astrocytes [12], and production of some chemical mediators in astrocytes [8,9,11], suggesting that ET_B receptors modulate pathophysiological responses of damaged brain. In this study, to clarify roles of brain ET_B receptors, the effects of an ET_B receptor agonist on AQP expression in the rat cerebrum were examined.

All experimental protocols conformed to the Guiding Principles for the Care and Use of Animals approved by the Japanese Pharmacological Society. Intracerebroventricular administration of $Ala^{1,3,11,15}$-ET-1 (Peninsula Labs, San Carlos, CA, USA), an ET_B agonist, was performed by the use of mini-osmotic pumps (Alzet2002, Durect Co., Cupertino CA, USA) and a 28-gauge stainless steel cannula brain infusion kit (Durect Co), as described before [12]. Briefly, a mini-osmotic pump was implanted in the subcutaneous tissue of male Wistar rats (250–300 g) under sodium pentobarbital anesthesia. The stainless steel cannula was inserted through a burr hole (position: 0.5 mm posterior and 2.0 mm right lateral from the bregma) into the cerebral ventricle (4.0 mm under the surface of the skull). $Ala^{1,3,11,15}$-ET-1 was dissolved in sterile artificial CSF (150 mM NaCl, 1.2 mM $MgSO_4$, 1 mM K_2HPO_4, and 10 mM D-glucose) and continuously administered at a dosage of

* Corresponding author. Tel.: +81 721 24 9462; fax: +81 721 24 9462.
E-mail address: koyamay@osaka-ohtani.ac.jp (Y. Koyama).

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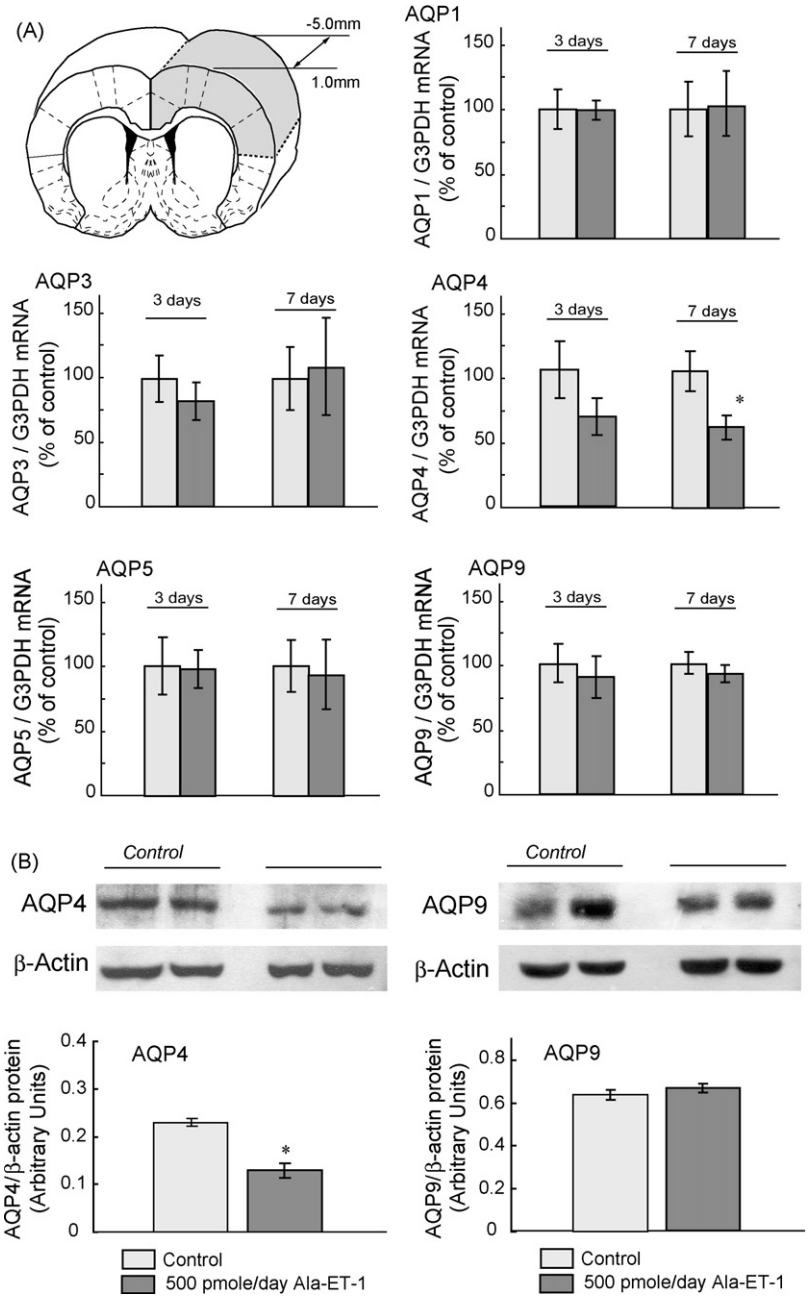

Fig. 1. Effects of intracerebroventricular administration of Ala^{1,3,11,15}-ET-1 on expression of AQPs in rat brain. (A) AQP mRNAs Either Ala^{1,3,11,15}-ET-1 (500 pmol/day) or artificial CSF (control) was infused into rat brain for 3 and 7 days. Total RNA was extracted from cerebral tissue obtained from the left hemisphere between 1.0 and −5.0 mm from the bregma (indicated as a shaded area in an illustration at the left upper). The copy numbers of AQP1, 3, 4, 5 and 9 mRNAs in the cerebrum were normalized to those of G3PDH. Results are means ± SEM of 7–9 rats and are presented as percentages of the level in control rats. $^{*}p < 0.05$ vs control by Student's *t*-test. (B) AQP proteins Ala^{1,3,11,15}-ET-1 (500 pmol/day) was infused into rat brain for 7 days. In each of control and Ala^{1,3,11,15}-ET-administration, typical immunoblots of cerebral AQP4 and AQP9 proteins from two different rats are presented. After detection of AQP4 and AQP9 proteins, the protein bands in X-ray films were subjected to densitometry analyses. The densities of AQPs were normalized to that of β-actin in the same blots. Results are means ± SEM of 5–6 rats and presented as ratios of AQP/β-actin proteins. $^{*}p < 0.05$ vs control rats by Student's *t*-test.

500 pmol/day. Control animals were infused with artificial CSF in the same manner. In some experiments, BQ788, an ET_B receptor antagonist, was included in the Ala^{1,3,11,15}-ET-1-containing artificial CSF and administrated at 5 nmol/day. After continuous administration of Ala^{1,3,11,15}-ET-1 for 3 or 7 days, rats were decapitated under deep ether anesthesia and the brains were removed. Cerebral tissue was obtained from the left hemisphere between 1.0 and −5.0 mm from the bregma (indicated by the illustration in Fig. 1A). Total RNA and tissue lysates were prepared from the brain tissue as described previously [8]. AQP mRNA levels were determined by real-time PCR using the SYBR Green fluorescent probe (Toyobo, Osaka, Japan) with the following primer pairs.

AQP1; 5′-CATGTATATCATCGCCCAGT-3′ and 5′-CACAGCCAGTGTAGTCAATG-3′,
AQP3; 5′-AGCAGATCTGAGTGGGCAGT-3′ and 5′-CTTGGGCTTAAGAGGGGAAC-3′
AQP4; 5′-GGTCAATGTCGATCACATGC-3′ and 5′-TTGGACCAATCATAGGCGC-3′
AQP5; 5′-TGGCCATAGGTACCTTAGCC-3′ and 5′-ACAGCCGGTGAAGTAGATCC-3′
AQP9; 5′-GATGCCTTCTGAGAAGGACG-3′ and 5′-AGAGAGCCATCACGACTGC-3′,
G3PDH; 5′-CTCATGACCACAGTCCATGC-3′ and 5′-TACATTGGGGGTAGGAACAC-3′.

As a standard for copy numbers of PCR products, serial dilutions of each PCR fragment were amplified in the same manner. Immunoblot analysis of tissue lysates prepared from rat cere-