Expression and localization of pituitary adenylate cyclase-activating polypeptide (PACAP) specific receptor (PAC1R) after traumatic brain injury in mice

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Summary. PACAP is a pleiotropic peptide and is well known to suppress neuronal cell death after ischemic injury through PACAP specific receptor (PAC1R). However, the pathological role of PAC1R is not elicited well on traumatic injured brain. Therefore, the purpose of this study is to investigate the expression and localization of PAC1R after traumatic brain injury (TBI) with immunohistochemistry. The PAC1R-immunoreactions (ir) were detected in peri-contusional area 3h after TBI and gradually increased up to 7d. Double immunohistochemical studies were revealed that the PAC1R-ir were co-localized with a microglial marker, CD11b. At 7d after TBI, the PAC1R-ir were merged with CD11b and with GFAP, an astroglial marker. These results suggested that PAC1R expresses in microglia and astrocyte with different time points after TBI, and PACAP and its receptor might play an important role for brain injury as well as ischemia.

Key words. PACAP, traumatic brain injury (TBI), controlled cortical impact (CCI), glial cells

1 Introduction

Traumatic brain injury (TBI) is a critical condition in the field of emergency medicine. In the westernized countries such as the United States and Japan, acute trauma death is the third major cause of death.

PACAP is a pleiotropic neuropeptide and belongs to the secretin/ glucagon/ vasoactive intestinal peptide (VIP) family. Several in vivo and in vitro studies have indicated that PACAP prevented neuronal cell death. PACAP binds to three receptors, two VIP/PACAP receptors (VPAC1R and VPAC2R) and PACAP specific receptor (PAC1R), which are seven transmembrane G protein-coupled receptors. PACAP can bind to PAC1R 1000-times higher affinity and it has been considering that PACAP prevents the neuronal cell death mediated by PAC1R (Arimura 1998). So far, it has been reported that the PAC1R is actively expressed in different neuroepithelia from early developmental stages and expressed in various brain regions during prenatal and postnatal development. However, it still remains as a controversial the role of PACAP and PAC1R on brain injury. In this study, we investigated the expression and localization of PAC1R after TBI of mice in a time-dependent manner.

2 Materials and Methods

Adult male C57/BL6 mice (Saitama, Saitama, Japan) were anesthetized with sodium pentobarbital (50mg/kg, ip).The animals were fixed on a stereotaxic frame and made carefully a burr hole on the left parietal bone with dental drill. Then, the animals were subjected to TBI by controlled cortical impact (a velocity of 5.82 m/s, duration of 47 ms, depth of 1.2 mm, and with a driving pressure of 73 psi) using an electrical compression device adapted for mice (eCCI Model 6.3; Custom Design, Richmond, VA). After injury, the mice perfused with saline followed by 2% PFA during 7 days, and prepared frozen blocks. All procedures involving animals were approved by The Institutional Animal Care and Use Committee of Showa University.

After immersed in H_2O_2, and treated with 10% normal goat serum (NGS), cryosections (8-μm) were then incubated in rabbit anti-PAC1R antibody (1:400, Suzuki et al. 2003) and were detected with biotinylated goat anti-rabbit IgG (1:200, Vector, Burlingame, CA) followed by ABC/DAB (Vector).

For double-staining, the sections (3 to 4 mice brains) were incubated with anti-PAC1R antibody (1:400) and any following primary antibody: mouse anti-GFAP (1:1000, Sigma, St Louis, MO); rat anti-CD11b (1:500, Serotec, Oxford, UK); mouse anti-NeuN (1:400, Chemicon, Temecula, CA) and

detected using Alexa fluoresence-labeled secondary antibodies followed by DAPI (1:10,000, Roche, Manheim, Germany).

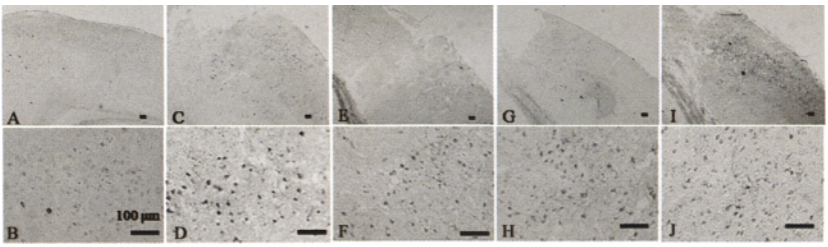

Fig. 1. Representative images of the PAC1R immunoreactions at 0 hour (A, B), 3 hour (C, D), 1 day (E, F), 4 days (G, H) or 7 days (I, J) after TBI. The PAC1R-ir was detected clearly in the perifocal area of the lesions at 3 hours after injury and expressed continuously during experimental periods. Scale bars = 100 μm.

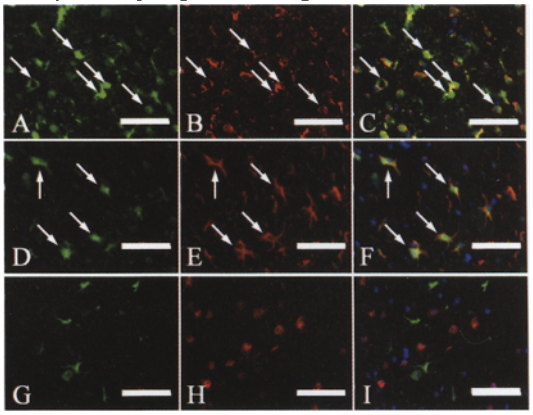

Fig. 2. PAC1R expressing cells 7d after TBI. PAC1R antibody (*green*) was co-stained with antibodies for CD11b (A–C), GFAP (D–F), or Neu N (G–I), respectively (*red*). Arrows are merged cells. Blue in C, F and I is DAPI nuclear staining. Scale bars = 50 μm.

3 Results and Discussion

PAC1R immunoreactions (ir) were detected in the perifocal area of the lesions from 3h after TBI (Fig. 1), and the intensity and number gradually increased up to 7d. PAC1R-expressing cells were identified at 1 and 7d in the perifocal area after TBI. Labelling with microglial (CD11b), astrtoglial (GFAP) or neuronal (NeuN) marker, the PAC1R-ir were merged with CD11b (+) cells, meaning microglia at 1d but no astrocytes and neurons were merged (data not shown). On the 7d, PAC1R-ir were co-localized with microglia and astrocytes, but not or less with neurons (Fig. 2).

In the present study, PAC1R was increased after TBI and expressed in

the microglia and astrocytes with different time point. PAC1R-ir were observed in the reactive astrocytes at 5d after a stab wound but not at 2d post surgery (Suzuki et al, 2003). We have also reported PAC1R expressed in neurons (Ohtaki et al, 2008). The diversity of PAC1R expressions could not explain only the differences of animal species. The PAC1R expressions might be different by the pathophysiological features of models such as inflammation and/or apoptosis. Further experiments will be needed to clarify these points. PACAP prevents post-ischemic neuronal cell death after ischemia. PACAP injection decreased hippocampus neuronal death along with an increase of interleukin-6 and inhibition of JNK and p38 phosphorylation after global ischemia (Dohi et al, 2002). Endogenous PACAP also played a critical role in the prevention of neuronal death after focal ischemia. PACAP decreased cytochrome c release from mitochondria by means of the regulation of bcl-2 (Ohtaki et al, 2008). The new insight of PAC1R expression and localization after TBI would contribute understanding for neuroprotective mechanisms of PACAP in brain pathology.

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