EFFERENT PROJECTIONS FROM MICTURITION SUPPRESSING REGION IN THE PERIAQUEDUCTAL GRAY OF THE MESENCEPHALON

Hypothesis / aims of study
The periaqueductal gray (PAG) of the mesencephalon has been implicated to be involved in the control of micturition [1]. Recent studies with positron emission tomography in humans showed an increase in regional cerebral blood flow in the PAG and cerebellum during urine storage [2, 3]. In cats, we reported the existence of micturition suppressing region in the dorsolateral margin of the rostral PAG (ICS 2006 in Christchurch). In the present study, we further investigated the efferent projections from micturition suppressing region in the PAG.

Study design, materials and methods
Nine decerebrated adult cats were used. To investigate the efferent projections from micturition suppressing region in the PAG, an antegrade neurotracer, biocytin, was injected into micturition suppressing region in the PAG. A microelectrode was inserted stereotaxically into the PAG and a region was sought where electrical stimulation (ES) suppressed isovolumetric bladder contractions. Micturition suppressing sites in the PAG were identified by ES only (ES group, 7 cats) or by ES and chemical stimulation (CS) with N-methyl-D-aspartate (ES+CS group, 2 cats). Six to 10 hours after the injection of the biocytin into micturition suppressing sites in the PAG, the animals were sacrificed and the brainstem was removed. Frozen slices were prepared, and biocytin was detected by reaction with streptavidin that has strong affinity for biocytin. Biocytin in the slices was reacted for streptavidin conjugated Alexa Flour®488 that is a fluorescent dye. These slices were examined with a fluorescence microscopy.

Results
Biocytin injection sites were recognized at the dorsolateral margin of the PAG in the 5 cats and at the ventral side of the PAG in the other 4 cats. Transported biocytin was detected at the pontine micturition center (PMC) in 2 of the 5 cats of the ES group, but was not detected at the PMC in the ES+CS group (Fig. 1). In all of the cats, biocytin was detected in the red nucleus and cerebellum (Fig. 2). In any cats, biocytin was not detected at the pontine storage center(PSC), micturition inducing sites of the PAG, or substantia nigra.

Interpretation of results
CS only activates neurons, while ES activates neurons and neural fibers of passage. Because biocytin was not detected at the PMC in the ES+CS group, the micturition suppressing region in the PAG is unlikely to have direct neural connections with the PMC. In addition, it seems unlikely that micturition suppressing region in the PAG suppresses micturition by directly inhibiting micturition inducing sites of the PAG, or by directly stimulating the PSC and substantia nigra. Because biocytin was detected at the red nucleus and cerebellum in all of the cats, micturition suppressing region in the PAG seems to have strong neural connections with these two sites in the central nervous system.

Concluding message
We assume that the PAG contains micturition suppressing region and its inhibitory action seems to be mediated via the red nucleus and cerebellum.

References
Fig. 1 Study for neural communication. A, B Schema of biocytin injection site in the PAG. Gray triangles are micturition suppressing sites identified by electrical stimulation (ES). Dark circles are micturition suppressing sites identified by ES and chemical stimulation (CS). C, D When biocytin was injected into the sites shown in A, transported biocytin was detected as white spots (arrows in D) medial to bilateral mesencephalic trigeminal tracts (MesV) in the tegmental pons. C, E When biocytin was injected into the sites shown in B, transported biocytin was detected ventral to bilateral MesV. The region that is ventral to MesV is corresponding to the PMC.

Fig. 2 Red Nucleus and cerebellum. A. Biocytin was diffusely detected at the bilateral red nucleus. B. In the cerebellum, biocytin was detected dominantly at the bilateral outer folia.

Specify source of funding or grant non
Is this a clinical trial? No
What were the subjects in the study? ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained? Yes
Name of ethics committee National Institutes of Health Guide for the Care and Use of Laboratory Animals and guidelines on the ethical use of animals in Asahikawa Medical College