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Topical Application of Substance P Facilitates Vestibular Functional Recovery Induced by AMPA in the Guinea Pig

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Abstract **Hypothesis:** Topical Substance P (SP) application facilitates recovery from vestibular disorders induced by (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) in guinea pigs and may offer a treatment strategy for patients with peripheral vestibular disorders. **Methods:** Sixteen Hartley white guinea pigs were assigned to groups administered with SP and artificial perilymph. A hole was drilled adjacent to a round window and AMPA was infused into the hole to induce a vestibular disorder. Thereafter, SP or artificial perilymph was delivered via an osmotic pump inserted into the hole. We observed spontaneous nystagmus and measured vestibulo-ocular reflexes (VOR) gains using sinusoidal rotation tests. **Results:** Spontaneous nystagmus decreased immediately after SP infusion, and the gain ratios were significantly higher than those in the control group at 3 and 7 days after treatment. **Conclusion:** Topical SP application facilitates recovery from AMPA-induced vestibular disorders in guinea pigs.

Key words: vestibular disease, substance P, vestibulo-ocular reflex, AMPA

Introduction

Substance P (SP), an excitatory neurotransmitter, is known to be extensively distributed in the central and peripheral nervous systems. In the 1980s and 1990s, high levels of SP in the peripheral vestibular apparatus were reported.[1-10] Although the role of SP in peripheral vestibular function has not yet been completely clarified, it has been shown that direct administration of SP into the unilateral inner ear resulted in excitation of the peripheral vestibule[18] and facilitated synaptic plasticity.[15] We hypothesized that treatment with SP would be useful in facilitating recovery of peripheral vestibular function. It is well known that glutamate induces excitotoxicity in neuronal cells in brain ischemia.[19] Topical application therapy is useful for treating inner ear diseases. The benefit of this therapy is that even drugs that cannot pass the blood-inner ear barrier can be administered in an adequate dose while avoiding systemic side effects. In this study, a model of partial vestibular dysfunction induced by topical application of (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) was developed.[21] SP was administered directly into the inner ears of guinea pigs with AMPA-induced vestibular disorders in order to evaluate its effect on vestibular function. This model is suitable for studying the pharmacological effects of candidate drugs for topical application therapy.

Methods

The study protocol was reviewed by the Yamaguchi University School of Medicine's Committee for Ethics in Animal Experiments. The study was conducted in accordance with the Guidelines for Animal Experiments of the Yamaguchi University School of Medicine, Law No. 105, and Notification

No. 6 of the Japanese government. Sixteen male Hartley white guinea pigs with normal tympanic membranes and Preyer reflexes were randomly assigned to receive constant intravestibular infusion of SP (10^{-3} M, n = 8) or artificial perilymph (113.5 mM NaCl, 5.4 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10.0 mM glucose, 10.0 mM N-2-hydroxyethylpiperazine N′-2-ethanesulfonic acid, n = 8) as a control.

Anesthesia was induced with a combination of xylazine (16 mg/kg, i.p.) and ketamine (16 mg/kg, i.p.), and 1.5 mL of lidocaine hydrochloride was injected into the right postauricular region for local anesthesia. Body temperature was maintained at 37 ℃. The mastoid bulla was opened by a postauricular incision to allow visualization of the round window using a surgical microscope (Carl Zeiss, Oberkochen, Germany). A hole was drilled adjacent to the round window using a perforating burr (Proxxon, 0.5 mm diameter; Kiso Power Tools, Osaka, Japan). A polyethylene catheter (0.2-mm inner diameter, 0.5-mm outer diameter; Natsume Co. Ltd., Tokyo, Japan) containing 10 mM AMPA (Sigma-Aldrich, St. Louis, MO, USA) connected to a syringe placed in a syringe pump (SP-70; Nipro Co., Osaka, Japan) was inserted into the hole and AMPA was infused at 0.6 mL/h for 5 min. Thereafter, the polyethylene catheter containing artificial perilymph was connected to an osmotic pump (Model 2002, Alza Corporation, Palo Alto, CA, USA) and inserted into the hole. The length of the polyethylene catheter was adjusted to deliver artificial perilymph over a period of 12 h. After the pump was positioned under the skin on the back of the animal, the wound was washed with saline. After closure, a small amount of piperacillin sodium (PIPC) (40 mg/kg) was injected intramuscularly, and oxytetracycline HCl ointment was applied to the wound. The body temperature of the animals was maintained at 37 ℃ throughout the procedure, and for 6 h thereafter, each animal was warmed by an electric blanket (Sanyo, Osaka, Japan). Thereafter the pump infused SP (12h after AMPA infusion).

Spontaneous nystagmus was observed throughout the experimental procedure. To measure the vestibulo-ocular reflexes (VOR), sinusoidal rotation tests were performed before and 3, 7, and 14 days after treatment. VOR gains were calculated using an in-house analysis system.[20] A cage designed to immobilize the guinea pigs during experiments was mounted on top of a turntable apparatus (Daiichi Medical, Tokyo, Japan). The head of each animal was firmly fixed with both auricles held between sponge-covered plates, and both acoustic meati were horizontally positioned so that the midpoint of a straight line joining the lateral semicircular canals was aligned with the rotation axis of the turntable. An infrared charge-coupled device CCD camera (Nagashima Medical, Tokyo, Japan) was positioned perpendicular to the sagittal plane of each guinea pig's head and parallel to the rotational plane of the turntable apparatus. By opening an aperture on the left side of the head cage, eye movements could be videotaped in the dark using the infrared CCD camera (mini DV format, Canon, Tokyo, Japan). Video images were stored on a Power Mac G5 (Apple Computer, Cupertino, CA, USA) and converted to image files using QuickTime (Apple Computer). A macro was created for use with Image J analysis software to automatically analyze guinea pig eye movements. Unnecessary portions were removed from the images of eye movements captured with the macro, and thresholds were then set to ensure clear outlines of the pupils. The X–Y center of each pupil was analyzed and horizontal and vertical components of the eye movements were calculated. Slow-phase velocities were calculated and the maximum slow-phase velocity was identified. The horizontal VOR gain was calculated by dividing the maximum slow-phase velocity by the peak angular velocity. Rotation testing was performed at 0.1 Hz, with a peak angular velocity of 60°/sec. To evaluate vestibular function, the gain ratio was defined as the ratio between the VOR gain on the treated side after treatment and the VOR gain on the treated side before treatment. Differences in gain ratios between groups on each examination day were evaluated using the Friedman test. The significance level was set at $P < 0.05$. All data are shown as means ± S.E.

Results

Spontaneous nystagmus decreased just after infusion (12 h after treatment) in the SP-treated group and significantly differed from that in the control group at 15 h after treatment (SP-treated group: 4.000 ± 2.430, control group: 17.297 ± 5.226; Fig. 1). Gain ratios were also significantly higher at 3 and 7 days after SP administration than in the control group (SP-treated group: 0.701 ± 0.057, control group: 0.972 ± 0.045; Fig. 2).

Discussion

The striking new finding in the present study is that topical SP application facilitated vestibular functional recovery from AM PA-induced toxicity. SP is expressed in the small cells of the vestibular ganglion[30] and abundantly present in the peripheral vestibular organ, particularly in the basal portions of the crista ampullaris,[7] which suggests that SP is released in the synaptic cleft between the hair cells and vestibular nerve endings. It has been reported that AMPA receptor is also present on the postsynaptic membrane of the hair cells and tends to be first disrupted in the current AMPA-applied experiment.[22] The present study clearly demonstrated that SP facilitates functional recovery from the AMPA-induced vestibular damage. Although the localization of neurokinin-1 (NK-1) receptor, which possesses high affinity for SP, has yet to be determined in the vestibular apparatus, SP is inferred to act on the postsynaptic membrane of the hair cells via the putative NK-1 receptor, as main targets.

In the present study, spontaneous nystagmus during the acute postoperative period was suppressed immediately after SP administration. This result may be attributable to the neurotrophic action or excitatory neurotransmission of SP. Furthermore, reduction in VOR was suppressed 3 days after the start of SP treatment. In our previous report, although SP was continuously administered in the perilymphatic space, the excitatory effect of SP was transient and disappeared after 24 h.[18] In this study, SP was administered

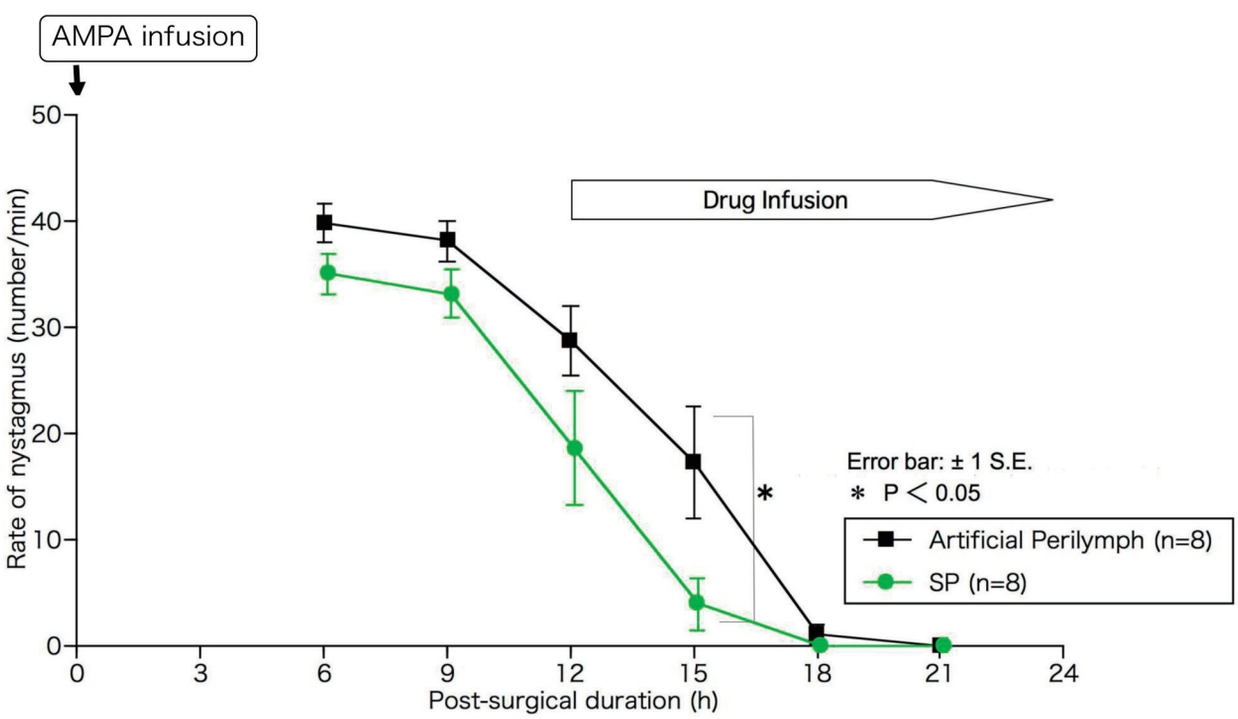

Fig. 1 Time course of the frequency of spontaneous nystagmus. The SP-treated group showed a tendency for a decrease in the frequency of nystagmus beginning immediately after the start of the medication.
*P < 0.05. Error bar indicates mean ± S.E.

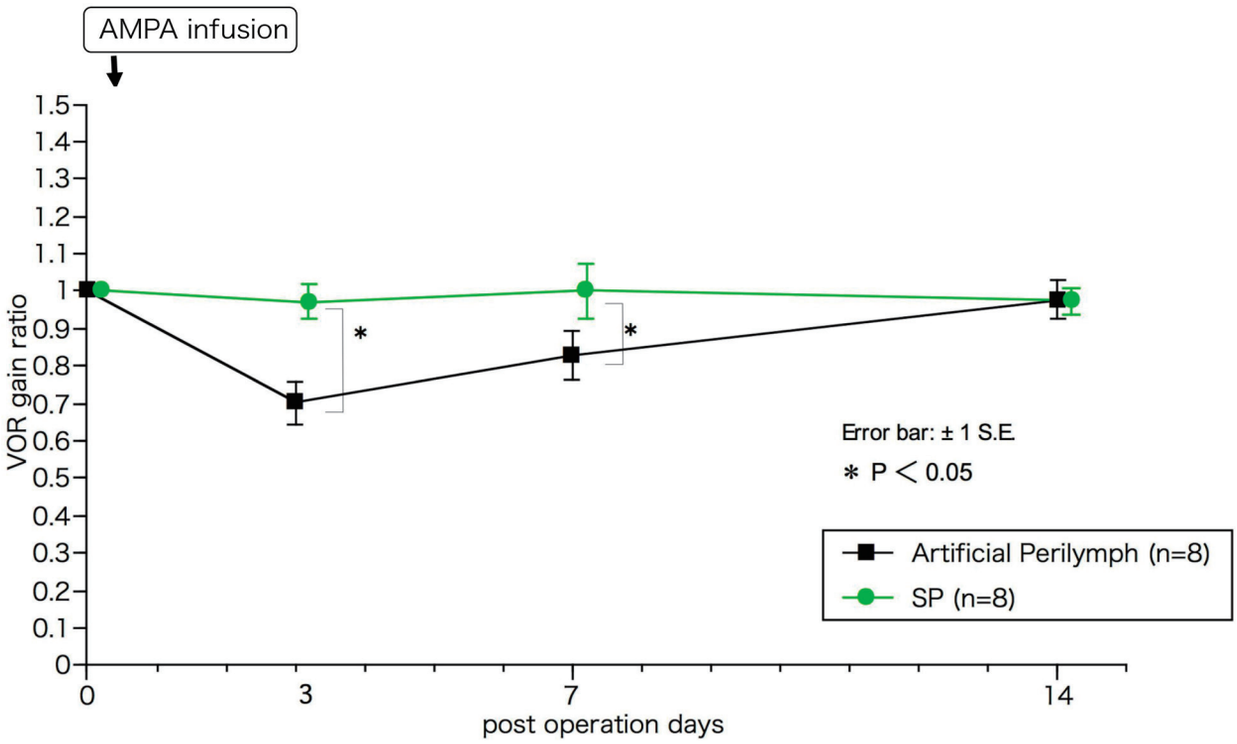

Fig. 2 Changes in the VOR gain ratio (the postoperative VOR gain divided by the preoperative VOR gain). In the SP-treated group, the reduction in the VOR gain ratio was significantly suppressed 3 days after the surgery.
*P < 0.05. Error bar indicates mean ± S.E.
VOR, vestibulo-ocular reflex

under the same conditions. Therefore, it is doubtful that SP maintains excitatory action for 3 days after the start of SP treatment.

Numerous reports have focused on the electrophysiological or neurotrophic effects of SP.[27-31] It has been shown that SP acts as an excitatory neurotransmitter or as a facilitatory neuromodulator on vestibular ganglion cells.[30,31] Furthermore, it has been reported that intratympanic administration of gentamicin induced increase in SP-like immunoreactivity in vestibular ganglion cells, suggesting the possibility that synthesis of SP protein was upregulated.[29] SP may act as a neurotrophic factor in damaged vestibular ganglion cells. AMPA is known to have an excitatory neurotoxic effect on neurons. It therefore seems probable that SP exerts neurotrophic effects against AMPA-induced disorders.

A previous study showed that intracochlear infusion of AMPA induces partial vestibular dysfunction predominantly by activation of AMPA receptors[21] localized in the postsynaptic region of vestibular hair cells.[22] When an excess of AMPA is applied topically, AMPA receptor activation induces excitotoxicity. When the damage is localized within the synapse, morphological and functional recovery can occur within 7 days.[23] When the damage is more severe, hair cells themselves may be involved, leading to cell death. The AMPA model presented here represents a reversible synaptic disorder, the data of which suggest that SP facilitates synaptic recovery. To verify the effects of SP, further morphological evidence is needed that SP treatment increases the number of regenerated synapses to the hair cells compared to the vehicle control.

In the current surgical procedures, particularly cochlear fenestration for drug delivery, vestibular function was demonstrated to recover within 2 weeks in all animals even in

the artificial-perilymph-treated group as previously reported.[24,25] Our infusion method is thus considered to cause no critical inner ear damage (i.e. by pressure injury), and require no effluent hole.

Several AMPA concentrations were evaluated with the infusion method described here. A relatively higher AMPA concentration was used than in previous studies.[23] Concentrations <10 mM caused no obvious static symptoms such as spontaneous nystagmus,[26] possibly because of the features of the infusion method. Because the catheter and cochlear hole is not tightly fit, perilymph leaks at the catheter insertion point after 2 min of infusion causing the AMPA concentration in the perilymphatic space to be <10 mM.

The findings of this study suggest that topical SP administration may be useful for treating patients with peripheral vestibular disorders.

Conflict of Interest

The authors state no conflict of interest.

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