Relationship between Arginine Vasopressin and High Signal Intensity in the Pituitary Posterior Lobe on T1-Weighted MR Images in Dogs

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ABSTRACT. The posterior lobe of the pituitary gland (PL) normally shows characteristic high signal intensity (SI) on T1-weighted MR images (T1WI) in humans. The high SI is thought to represent storage of arginine vasopressin (AVP) in the PL. Normal dogs also show a high SI on T1WIs, but the origin is unclear. In the present study, we investigated whether the high SI in the PL on T1WIs in normal dogs is caused by AVP. We examined the SI in the PL on T1WIs, plasma AVP concentrations and plasma osmolality in normal dogs after excessive AVP secretion was induced by hypertonic saline overload. In addition, functional changes in the supraoptic nucleus and paraventricular nucleus of the hypothalamus under AVP secretion-stimulated conditions were examined immunohistologically. Under hypertonic saline overload, plasma osmolality and plasma AVP concentrations gradually increased, while the SI of the PL gradually decreased. This suggests that AVP secretion was stimulated by elevated osmolality. Moreover, there was a significant negative correlation between plasma AVP concentrations and the SI ratio of the PL. An immunohistochemical study of the hypothalamus nucleus revealed that AVP-immunopositive cells significantly increased in the hypertonic saline loaded dogs. We concluded that the high SI in the PL in T1WIs in normal dogs was caused by AVP stored at the site, and examination of the SI in the PL using MRI is useful for diagnosis of abnormal pituitary glands.

KEY WORDS: arginine vasopressin, canine, MRI, pituitary, signal intensity.

Magnetic Resonance Imaging (MRI) provides detailed anatomic information and is a useful diagnostic modality for a variety of diseases in human and veterinary clinical medicine. In particular, the utility of the MRI has been recognized in examinations of the central nervous system. It has been used not only to examine morphological changes in the hypothalamic-neurohypophyseal (HNS) system, but also to evaluate function by examining signal intensity in images [3]. For these reasons, the MRI is the first-choice modality for evaluation of HNS disease.

The posterior lobe of the normal pituitary shows characteristically high signal intensity (SI) in T1-weighted images (T1WI) at two months after birth or later in humans, which differs from the anterior lobe of the pituitary [2, 6, 20]. In addition, the characteristically high SI is markedly decreased by depletion of arginine vasopressin (AVP) due to disorders such as central diabetes insipidus [8, 17–19]. On the basis of this change in T1WIs, the MRI is used for diagnosis of diabetes insipidus [16], ectopic posterior lobe [9, 24] and depleted posterior lobe of the pituitary by continuous excessive AVP secretion due to diabetes mellitus [5]. Moreover, the presence and localization of the characteristically high SI (so-called “pituitary flush”) can be helpful in diagnosing microadenoma in dogs with pituitary-dependent hyperadrenocorticism without obviously enlarged pituitaries [23]. Although the posterior lobe of the normal pituitary in dogs exhibits hyperintensity in T1WIs as observed in humans, the cause of the hyperintensity has not been investigated.

In this study, we investigated whether the high SI in the posterior lobe of the pituitary (PL) based on T1WIs in normal dogs is caused by AVP. We examined the relationship between SI in the PL based on T1WIs and the concentrations of AVP circulating in the blood of normal Beagles after excessive AVP secretion was induced by hypertonic saline (HS) overload. Functional changes in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus under AVP secretion-stimulated conditions were examined histologically.

MATERIALS AND METHODS

Animals: Twelve healthy Beagles (5 males and 7 females), aged 1–3 years (mean: 2.5 years old), weighing 10.0–15.0 kg (mean: 11.9 kg), and having normal values for complete blood count, blood chemistry, blood gas analysis and serum electrolyte tests were randomly divided into control (n=6; 2 males and 4 females), and HS groups (n=6; 3 males and 3 females).

Experimental design: Anesthesia was induced with thiopental Na. The dogs in the HS group were intubated, and anesthesia was maintained with isoflurane (1.5–2.0%) in

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oxygen and spontaneous ventilation. Under general anesthesia, HS (10% NaCl; Otsuka Chemical Co., Ltd.) was administered from a right cephalic vein at a rate of 0.12 ml/kg/min for 90 min according to the method of Biewenga et al. with some modifications [1]. Before HS administration and at 30, 60, and 90 min after HS administration, the plasma AVP concentrations, plasma osmolality and SI ratio in the PL were measured. After anesthesia was introduced, the dogs received Lactated Ringer’s solution continuously at a rate of 5 ml/kg/hr. Four dogs in the HS group were euthanized immediately after MRI at 90 min after HS administration, and hypothalamus tissue was excised for histological examination.

In the control group, three dogs underwent general anesthesia, and the plasma AVP concentrations, plasma osmolality and SI ratio in the PL were measured at 0, 30, 60 and 90 min after anesthesia was induced. Subsequently, six animals in the control group were euthanized, and hypothalamus tissue was excised.

The experimental protocol was approved by the Committee on Bioethics of Nippon Veterinary and Life Science University.

**MRI and measurement of the SI ratio:** In the HS group, all dogs underwent an MRI under general anesthesia before HS administration and at 30, 60, and 90 min after HS administration. MRIs were performed using a 1.5 Tesla superconducting magnet (VISART, Toshiba Medical System Corporation, Tokyo, Japan). In order to include the pituitary, sagittal T1WIs of the midline of the brain and axial T1WIs perpendicular to the bottom of the cranium were taken by the spin-echo method under the following conditions: (1) a TR/TE of 350/15 msec, slice thickness of 2 mm without an interslice gap, 160 × 256 matrix, field-of-view of 8 cm, acquisition of four signals and an imaging time of 7 min 32 sec for Sagittal images and (2) a TR/TE of 400/15 msec, slice thickness of 2 mm without an interslice gap, 160 × 256 matrix, field-of-view of 8 cm, acquisition of four signals and an imaging time of 8 min 32 sec for Axial images. The sagittal and axial images with the largest areas of the posterior lobe were used for measurement of SI. SI was measured three times each at the PL and pons from a sagittal image and at the PL and cerebral cortex from an axial image, and the SI ratio of the PL was calculated from the sagittal and axial images as follows: Sagittal SI (Sls) = average SI of the PL divided by average SI of the pons; Axial SI (Sla) = average SI of the PL divided by average SI of the cerebral cortex [13, 14].

Sls and Sla were determined in the three dogs in the control group that underwent MRI at 0, 30, 60, and 90 min after anesthesia was induced.

**Measurement of AVP concentrations:** Prior to MRI, blood was collected from the left cephalic vein to measure plasma osmolality and plasma AVP concentrations. The blood samples for measurement of plasma osmolality and plasma AVP concentrations were treated with ethylenediaminetetraacetic acid, and the plasma was separated by centrifugation under cooled conditions (4°C at 1,470 G for 15 min). Plasma AVP concentrations were measured using the RIA double antibody technique, while plasma osmolality was determined using the cryoscopic method.

**Immunohistochemical staining:** After sacrifice, the hypothalamus tissue was harvested as a 15 × 15 × 15 mm cube so that it would contain the third cerebral ventricle at the center of the cube. The cube was cut into 4 blocks at the frontal plane. The blocks were then fixed in 4% PFA at 4°C for 24 hr, embedded in paraffin and thin-sectioned. As described in a previous report, the thin-sectioned tissues were stained immunohistochemically using the peroxidase-dase-labeled antibody method. Anti-AVP antibody (rabbit anti-human vasopressin, Biomeda Corp.) was used [22]. After staining, AVP-immunopositive cells were counted in the SON and PVN of the hypothalamus in each section, and the largest number of immunopositive cells was adopted.

**Statistical analysis:** In the control and HS groups, plasma osmolality, plasma AVP concentrations, Sls and Sla were compared by one-way repeated measures ANOVA followed by Tukey-Kramer post-hoc test for comparison between before and at 30, 60, and 90 min. In addition, plasma osmolality and plasma AVP concentrations at each point were compared between the control and HS groups using Mann-Whitney’s U test. The associations between plasma osmolality and plasma AVP concentrations and between SI ratio and plasma AVP concentrations were examined by Pearson’s correlation coefficient. The number of AVP-immunopositive cells in the hypothalamus in the control and HS groups, the numbers in the SON and PVN and the sum of the two were compared by Student’s t-test. In these comparisons, p<0.05 was regarded as significant.

**RESULTS**

**Physiological parameters:** The changes in plasma osmolality and plasma AVP concentrations are shown in Fig. 1-A, B. In the HS group, plasma osmolality before HS administration was 290.5 ± 5.3 mOsm/l (mean ± SD). After HS administration, plasma osmolality gradually increased significantly (30 min, 320.3 ± 4.6 mOsm/l, p<0.05; 60 min, 338.7 ± 12.8 mOsm/l, p<0.05; 90 min, 347.2 ± 10.3 mOsm/l, p<0.05). In addition, the plasma AVP concentrations were 3.94 ± 2.42 pg/ml before HS administration, and they increased significantly in a similar manner to the plasma osmolality (30 min, 13.0 ± 3.8 pg/ml; 60 min, 23.5 ± 7.7 pg/ml, p<0.05; 90 min, 40.1 ± 14.6 pg/ml, p<0.05). In the HS group, the plasma osmolality and plasma AVP concentrations increased significantly at 30, 60 and 90 min compared with those of the control group (p<0.05, respectively). Furthermore, in the HS group, the plasma AVP concentrations were positively and significantly correlated with plasma osmolality with a correlation coefficient of r=0.757 and a regression line of Y=-157.047+0.545X (p<0.05; Fig. 2-A).

In the control group, the plasma AVP concentrations immediately after introduction of anesthesia was 5.27 ± 2.26 pg/ml, and it gradually increased significantly (30 min, 8.1 ± 1.6 pg/ml; 60 min, 12.9 ± 2.2 pg/ml, p<0.05; 90 min, 19.8 ±
3.5 pg/ml, p<0.05; Fig. 1-B). However, in the control group, the plasma AVP concentrations did not correlate with the plasma osmolality (a correlation coefficient of r=0.125, p=0.71; Fig. 2-B).

Changes in the SI ratio: A high SI was observed at the PL in all dogs before HS administration and immediately after anesthesia was induced (Fig. 3). The sequential changes in the SI ratio are shown in Fig. 4-A, B. Before administration in the HS group, SIs and SIa were 2.55 ± 0.37 and 2.18 ± 0.23, respectively, and they gradually decreased after administration (2.37 ± 0.32 at 30 min, 2.16 ± 0.38 at 60 min and 2.10 ± 0.35 at 90 min for SIs and 2.09 ± 0.39 at 30 min, 1.97 ± 0.32 at 60 min and 1.79 ± 0.32 at 90 min for SIa; p<0.05). In the control group immediately after anesthesia was induced, SIs and SIa were 2.52 ± 0.17 and 2.32 ± 0.17, respectively, and they gradually decreased after anesthesia (2.45 ± 0.30 at 30 min, 2.25 ± 0.16 at 60 min and 2.24 ± 0.30 at 90 min for SIs and 2.32 ± 0.17 at 30 min, 2.18 ± 0.72 at 60 min 2.10 ± 0.28 at 90 min for SIa).

Correlation between plasma AVP concentrations and the SI ratio: With regard to the plasma AVP concentrations and SI ratio of the PL, SIs revealed a correlation coefficient of r=0.383 and a regression line of Y=54.093–15.895X (p<0.05), while SIa showed a correlation coefficient of r=0.570 and a regression line of Y=72.477–26.615X (p<0.05). The plasma AVP concentrations showed a significant negative correlation with SIs and SIa (Fig. 5-A, B).

AVP-immunopositive cell counts: The AVP-immunopositive cells detected in the hypothalamus using anti-AVP antibodies in the HS and control groups are shown in Fig. 6. The AVP-immunopositive cells in the SON and PVN were counted. The number of AVP-immunopositive cells in the SON was 198.7 ± 37.8 cells/slice in the control group and 234.5 ± 31.9 cells/slice in the HS group. In the PVN, the number of AVP-immunopositive cells was 134.0 ± 42.2 cells/slice for the control group and 169.0 ± 37.4 cells/slice for the HS group. There was a significant difference in the sum of AVP-immunopositive cells in the SON and PVN.
between the control and HS groups (p<0.05; Fig. 7).

DISCUSSION

In 1984, Mark et al. reported that high SI in the PL on T1WIs is caused by a “fat pad” present in the pituitary fossa [15]. Later, according to studies on humans and rabbits, the characteristically high SI in T1WIs of the PL is attributed to storage of AVP [6, 8, 11, 12]. Although the normal PL in dogs shows a high SI by T1WI as in humans and rabbits, no study has shown that AVP stored at the site in the cause of high SI.

A variety of regulatory mechanisms have been demonstrated for AVP secretion [4]. AVP secretion is stimulated under general anesthesia with isoflurane because of a decrease in blood pressure due to dilation of peripheral vessels. In addition, changes in plasma osmolality have been shown to cause secretion of AVP, and elevation of plasma osmolality stimulates both synthesis and release of AVP.

Herein, we attempted to induce excessive AVP secretion into peripheral blood from the PL by stimulation with HS. Administration of HS to normal beagles resulted in significant sequential elevation of plasma osmolality and plasma AVP concentrations. Moreover, in the HS group, there was a significant positive correlation between the plasma osmolality and plasma AVP concentrations, but not in the control

Fig. 3. Sequential changes in SI in the PL in T1WIs. Sagittal and Axial views in T1WIs for the HS group. Normal high SI in the PL is well demonstrated before HS administration (arrow; a, c). T1WIs obtained at 30 min (b, f), 60 min (c, g) and 90 min (d, h) after HS administration. SI in the PL gradually decreased after HS administration.

Fig. 4. Changes in SIs and SIa. Closed circle: the control group; closed square: the HS group. (A) The graph shows sequential changes in SIs. *: In the HS group, SIs decreased significantly at 60 and 90 min after administration of HS compared with that before administration (p<0.05). (B) The graph shows sequential changes in SIa. *: In the HS group, SIa decreased significantly at 90 min after administration of HS compared with that before administration (p<0.05).
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Fig. 5. Correlation between SI and plasma AVP concentrations. (A) A significant correlation can be seen between SIs and the plasma AVP concentrations ($r=0.383, p<0.05$). (B) A significant correlation can be seen between SIsa and the plasma AVP concentrations ($r=0.570, p<0.05$).

Fig. 6. Immunohistochemistry of the hypothalamus. Immunohistochemistry using anti-AVP antibody at the paraventricular nucleus (a) and supraoptic nucleus. *: Third cerebral ventricle.

Fig. 7. Comparison of the numbers of AVP-immunopositive cells. SON: supraoptic nucleus; PVN, paraventricular nucleus; Total, the sum of AVP-immunopositive cells in the SON and PVN. Open bar, the control group; closed bar, the HS group. There was a significant increase in the sum of AVP-immunopositive cells in the HS group compared with that of the control group ($p<0.05$).

group, suggesting that sequential elevation of the AVP concentration was caused by AVP secretion due to elevation of osmolality.

Although it is possible to measure absolute CT values from CT images on the basis of the difference in X-ray absorption speed in tissue, it is impossible to determine absolute values by MRI. According to previous reports on the SI of the PL [13, 14], we measured SI at the cerebral cortex and pons using the images taken to measure SI in the PL and investigated the changes in SI in the PL by calculating the relative ratio of SI at the site. There was a gradual decrease in the SI ratio in the PL in the HS and control groups. In addition, there was a significant negative correlation between the SI ratio and plasma AVP concentrations. We presumed that the release of AVP from neuroendocrine granules decreased AVP storage in the PL and decreased the SI ratio. Consistent with the results in this study of the correlation between plasma AVP concentrations and the SI ratio in normal Beagles, previous reports have shown reduction of the SI in the PL by oral administration of 2% NaCl [7] and a correlation between plasma AVP concentrations...
and SI in the PL under water deprivation conditions [14]. AVP is synthesized by magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus. AVP, a binding carrier protein (neurophysin), and a glycoprotein (copeptin) are enveloped by neurosecretory granules. The neurosecretory granules are transported down the axon and stored in the nerve terminals in the posterior lobe. During exocytosis, AVP is separated from neurophysin [7, 17, 21]. Currently, high SI in the PL in humans and rabbits is thought to be caused by the AVP-neurophysin complex stored in the PL [7, 10, 13, 21]. Our results show reduction of high SI in the posterior lobe in the HS and control groups, but no image change in the hypothalamus. However, immunohistochemistry demonstrated a significant increase in the number of AVP-immunopositive cells present in the SON and PVN where AVP was produced in the HS group compared with the results from the control group. In addition, AVP stored in the PL decreased remarkably in the HS group, and an increase in AVP production in the SON and PVN resulted in a significant increase in AVP-immunopositive cells.

In conclusion, our results demonstrated that high SI in the PL in T1WIs of normal dogs was caused by the AVP stored at the site. Since there was a significant correlation between the plasma AVP concentrations and the SI ratio in the PL, examination of high SI in the PL using MRI is helpful in diagnosis of abnormal pituitary glands, such as central diabetes insipidus and pituitary microadenomas.

REFERENCES

