Involvement of Endogenous Vasopressin in High Plasma Osmolality-Induced Anorexia via V1 Receptor-Mediated Mechanism

Ryota IKEMURA1), Takashi MATSUWAKI1), Keitaro YAMANOUCHI1) and Masugi NISHIHARA1)*

1) Department of Veterinary Physiology, Veterinary Medical Science, The University of Tokyo, Tokyo 113–8657, Japan
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ABSTRACT. It is known that water deprivation or injection of hypertonic saline induces anorexia. The present study examined the possible involvement of vasopressin in the suppression of food intake during high plasma osmolality. Intraperitoneal injection of vasopressin (20 µg/kg) into male rats significantly suppressed food intake for 1 hr. This anorectic effect of vasopressin was reversed by simultaneous injection of a peptide antagonist for V1 receptor (40 µg/kg), but not for V2 receptor (40 µg/kg). Intraperitoneal injection of hypertonic saline (20% NaCl, 2 ml/kg) similarly suppressed food intake for 2 hr, which was associated with a transient increase in plasma vasopressin concentrations. This hypertonic saline-induced suppression of food intake was blocked by a V1 receptor antagonist. Vasopressin (40 ng/2 µl) directly administered into the third ventricle of the brain also suppressed food intake for 1 hr. These results suggest that vasopressin participates in the suppression of food intake during high plasma osmolality, the action of which is mediated by V1 receptors in the brain.

KEY WORDS: food intake, plasma osmolality, vasopressin, V1 receptor.

The mechanisms regulating appetite attract attention in recent years due to the increasing risks of appetite disorders, obesity and related metabolic diseases [25, 26]. Appetite is recognized to be primarily controlled by the hypothalamus and many other factors are involved in the regulation; including peptides derived from peripheral tissues such as leptin [12] and ghrelin [19], as well as energy substrates such as glucose and fatty acids [9]. In addition, it has been suggested that food intake is also influenced by plasma osmolality and volume. For example, during high plasma osmolality caused by water deprivation [4] or injection of hypertonic saline [8], food consumption is decreased. Although the mechanism underlying the suppression of food intake by high plasma osmolality is not well understood, vasopressin might be involved because intraperitoneal injection of vasopressin has been shown to decrease food consumption in rats [18].

The secretion of vasopressin is controlled by plasma osmolality and also affected by blood pressure, blood volume, angiotensins and stress [17]. Vasopressin stimulates water absorption in renal collecting ducts, raises blood pressure and elicits corticotropin (ACTH) release from the anterior pituitary synergistically with corticotrophin-releasing hormone (CRH) from the hypothalamus [17, 22, 23]. There are three vasopressin receptors that mediate vasopressin effects, i.e., V1a, V1b and V2 receptors [1]. V1 receptors are localized in both the central nervous system and peripheral tissues [2, 10, 21], while V2 receptors are primarily localized in the kidney where they mediate the antidiuretic effect of vasopressin [21]. The anorectic effect of exogenously administered vasopressin was shown to be reversed by a V1 receptor antagonist [18].

In the present study, to elucidate whether endogenous vasopressin is indeed involved in the suppression of food intake during high plasma osmolality, the effect of a vasopressin receptor antagonist on food intake following the injection of hypertonic saline was investigated. Further, to test the possibility that vasopressin acts directly on the brain to induce anorexia, the effect of intracerebroventricular injection of vasopressin on food intake was examined.

MATERIALS AND METHODS

Animals: Adult (350–450 g) male Wistar-Imamichi rats (Imamichi Institute for Animal Reproduction, Tsuchiura, Japan) were housed individually in the hanging metal cages. All rats were given pellet food (MR Breeder, Nihon-Nosan, Japan) and tap water ad libitum. The animal room was maintained at 22 ± 2°C with a 12:12 hr light-dark cycle (lights on at 21:00). The experiments were conducted according to the guidelines for the care and use of laboratory animals, Graduate School of Agriculture and Life Sciences, the University of Tokyo.

Reagents: [Arg8]-vasopressin, [β-mercaptopo-β, β-cyclopentamethylenelepro-pionyl]1, O-methyl-Tyr5, Arg8]-vasopressin (V1 receptor antagonist), and [adamantaneacetyl1, O-ethyl-D-Tyr2, Val4, aminobutyryl6, Arg8,9]-vasopressin (V2 receptor antagonist) were obtained from Sigma (Saint Louis, MO, U.S.A.).

Hormone assay: Plasma vasopressin concentrations were measured by [Arg8]-vasopressin enzyme immunoassay kit (Assay Designs, Inc., Ann Arbor, MI, U.S.A.) according to the manufacturer’s protocol. The assay sensitivity was 3.4 pg/ml, and the intra- and interassay coefficients of variation were 8.9% and 7.0%, respectively.

*Correspondence to: NISHIHARA, M., Department of Veterinary Physiology, Veterinary Medical Science, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan.
Experiment 1: To examine whether the suppressive effect of vasopressin on food intake is mediated by V1 or V2 receptors, vasopressin (20 µg/kg) was intraperitoneally injected alone or in combination with a V1 antagonist (40 µg/kg) or a V2 antagonist (40 µg/kg) at the start of dark phase (9:00). All the drugs were dissolved in 0.9% NaCl solution, and injection volume was adjusted to be 2 ml/kg body weight irrespective of drug concentrations. Control rats received physiological saline (0.9% NaCl; 2 ml/kg). Food and water intake was measured 1, 2 and 3 hr after the injection.

Experiment 2: To examine if endogenous vasopressin is involved in the suppression of food intake, hypertonic saline (20% NaCl) was intraperitoneally injected alone or in combination with a V1 antagonist (40 µg/kg) at 9:00. The injection volume was adjusted to be 2 ml/kg body weight. Food and water intake was measured 1, 2 and 3 hr after the injection. In addition, blood samples were collected by decapitation 0, 15, 30 and 60 min after the injection of 0.9% or 20% NaCl solution, and plasma vasopressin concentrations were measured as described above.

Experiment 3: To elucidate if vasopressin could directly act on the brain to suppress food intake, the effect of intracerebroventricular injection of vasopressin on food intake was examined. Rats were anesthetized with sodium pentobarbital (40 mg/kg), and the cannula was placed into the 3rd ventricle using stereotaxic apparatus (Narishige, Tokyo, Japan). The coordinates for the tip of the cannula were 7.2 mm anterior from the lambda, 8.5 mm below the dura, and 0.0 mm from the midline. After the recovery period of at least 1 week, rats were given intracerebroventricular injection of either physiological saline (2 µl) or vasopressin (40 ng/2 µl) under the free moving condition at 9:00. Food and water intake was measured at 1, 2 and 3 hr after the injection.

Statistics: The data were analyzed by one way functional ANOVA followed by Fishers PLSD as a post-hoc test. Differences at P<0.05 were considered statistically significant.

RESULTS

Experiment 1. Effect of V1 and V2 antagonists on vasopressin induced-suppression of food and water intake: Intrapertitoneal injection of vasopressin significantly suppressed food and water intake for 1 and 3 hr, respectively (Figs. 1 and 2). The V1 antagonist used completely restored the suppressive effect of vasopressin on both food and water intake (Fig. 1), while the V2 antagonist did not affect the vasopressin effects (Fig. 2). Although the V1 antagonist alone affected neither food nor water intake (Fig. 1), the V2 antagonist increased water intake for 2 hr (Fig. 2).

Experiment 2. Effect of V1 antagonist on hypertonic saline induced-changes in food and water intake: Following intraperitoneal injection of hypertonic saline, plasma vasopressin concentrations transiently increased and returned to control levels 30 min after the injection (Fig. 3). The injection of hypertonic saline decreased food intake for 2 hr and increased water intake for 1 hr (Fig. 4). Simultaneous injection of the V1 antagonist restored food intake during 1–2 hr period without affecting water intake.

Experiment 3. Effect of intracerebroventricular injection of vasopressin on food and water intake: The injection of vasopressin into the 3rd ventricle significantly decreased food intake for 1 hr, while it did not affect water intake (Fig. 5).

DISCUSSION

The present study demonstrated that intraperitoneal injection of vasopressin suppressed food intake, and that this anorectic effect of vasopressin was reversed by a V1 receptor antagonist. These observations were consistent with
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those by Langhans et al. [18]. We further showed in this study that a V₂ receptor antagonist did not affect the suppressive effect of vasopressin on food intake, suggesting that vasopressin’s anorectic effect is solely mediated by V₁ receptors. The V₂ receptor antagonist alone increased water intake without affecting food intake, while the V₁ receptor antagonist alone affected neither food nor water intake. Since V₂ receptors are primarily present in the kidney, but not in the brain [21], the effect of the V₂ receptor antagonist on water intake may result from dehydration due to the blockade of the antidiuretic effect of vasopressin on the collecting duct of the kidney. On the other hand, the suppression of water intake by vasopressin alone observed in the present study may at least partially result from its antidiuretic effect.

Both high plasma osmolarity and hypovolemia were shown to decrease food intake as well as elicit vasopressin release [8, 24]. In the present study, intraperitoneal injection of hypertonic saline suppressed food intake, which was associated with a transient increase in plasma vasopressin concentration. The blockade of vasopressin actions by the V₁ receptor antagonist reversed the effect of high plasma osmolarity on food intake, indicating that endogenous vasopressin indeed plays a crucial role in suppressing food intake via V₁ receptors under these circumstances. The observation that, while food intake was suppressed up to 2 hr, plasma vasopressin concentrations returned to the control levels within 30 min after the injection of hypertonic saline suggests that the anorectic effect of vasopressin was not direct but rather indirect by being mediated by other hypothalamic factors controlling appetite [15]. However, since we did not measure vasopressin levels in the hypothalamus, the possibility that vasopressin induced anorexia by acting directly on the hypothalamic neuronal system controlling food intake cannot be ruled out.

In the present study, intracerebroventricular administration of vasopressin suppressed food intake without affecting water intake. This suggests that vasopressin exerts the anorectic effect through V₁ receptors in the brain [2], while it affects water intake through V₂ receptors in the kidney as mentioned above. Since there are abundant nerve terminals containing vasopressin within the hypothalamus [3, 11], vasopressin released as a neurotransmitter or neuromodulator probably plays a role in the hypothalamus. This notion is further supported by the previous report suggesting that vasopressin hardly penetrates the blood-brain barrier [16]. In the hypothalamus, V₁₅ receptors were mainly localized in
the suprachiasmatic nucleus, arcuate nucleus and lateral hypothalamic area [2], while most intense V₁b receptor-immunoreactivity was observed in the median eminence [10]. Among these hypothalamic regions, the arcuate nucleus and lateral hypothalamic area are well known to participate in regulating food intake [15]. Taken together, the anorectic effect of vasopressin may be at least partially exerted via V₁ receptors in the arcuate nucleus and lateral hypothalamic area.

The secretion of vasopressin has been shown to be stimulated by a wide variety of stress [7, 13, 20] as well as hyperosmolality and hypovolemia [8], and the stimulatory effect of vasopressin on ACTH release from the anterior pituitary is well documented [22, 23]. Under stress conditions, food intake is suppressed in common, which is at least partially attributed to the increase in the release of α-melanocyte-stimulating hormone through the activation of CRH neurons [5, 6, 14, 15]. Vasopressin may also participate in inducing anorexia during stress synergistically with CRH.

In conclusion, the present study suggests that vasopressin is involved in suppressing food intake during high plasma osmolality via V₁ receptors in the brain. Since a large amount of water needs to be secreted into the gastrointestinal tract for the digestion of ingested food, vasopressin may cause anorexia and thereby coordinates body fluid balance and food intake under dehydrated conditions.

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