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Activation of Ventrolateral Medulla Neurons by Arginine Vasopressin *via* V_{1A} Receptors Produces Inhibition on Respiratory-Related Hypoglossal Nerve Discharge in the Rat

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Abstract

Arginine vasopressin (AVP) is an important neurohormone in the regulation of many aspects of central nervous system, yet its modulation on the respiratory function remains largely unknown. The aims of this study were to investigate the modulation of phrenic (PNA) and hypoglossal nerve activity (HNA) by central administration of AVP and to identify the involvement of AVP V_{1A} receptors in this modulation. Animals were anesthetized with urethane (1.2 g/kg, i.p.), paralyzed with gallamine triethiodide (5 mg/kg, i.v.), and artificially ventilated. The rat was then placed on a stereotaxic apparatus in a prone position. PNA and HNA were monitored at normocapnia in hyperoxia. Microinjection of AVP into the medial ventrolateral medulla (VLM) and/or rostral ventral respiratory group (rVRG) produced a dose-dependent inhibition on both PNA and HNA, whereas the microinjection of AVP into the region of lateral VLM resulted in a similar inhibition of these nerve activities and a pressor response. Systemic administration of phentolamine abolished the pressor effect but did not affect the inhibition of PNA and HNA evoked by AVP injection into the lateral VLM and/or rVRG, suggesting that AVP-induced inhibition of PNA and HNA was not due to the side effect of pressor response. These cardiopulmonary modulations were totally abolished by the central pretreatment of AVP V_{1A} receptor antagonist. Our results suggested that AVP may activate neurons located at the VLM and/or rVRG *via* the AVP V_{1A} receptor to inhibit respiratory-related HNA and thus to regulate upper airway aperture.

Key Words: arginine vasopressin (AVP), AVP V_{1A} receptor antagonist, ventrolateral medulla, phrenic nerve activity, hypoglossal nerve discharge, blood pressure, rats

Introduction

The hypoglossal nerves (HN) innervate the genioglossus muscle (GG), which is the main body of the tongue. The tongue is involved in both somatic activities and visceral functions, such as swallowing, chewing, phonation, and food intake (18, 19, 34). It also displays respiratory-related discharges during the respiratory cycle (11). Inspiratory discharges of the HN and/or the GG will protrude the tongue and widen the anterioposterior diameter of the oropharynx, which, in turn, reduce the resistance to airflow through the upper airway (UAW). In contrast, a decrease or loss of this inspiratory discharge of the HN and GG will result in a decrease in oropharygneal diameter, and even collapse the oropharynx, as seen in patients with obstructive sleep apnea (OSA) (8). Many physiological factors play a modulatory effect on phasic hypoglossal nerve activity (HNA). Hypercapnia, hypoxia, and changes in pressure or airflow within the UAW produce an increase in HNA (11, 12, 13, 9, 29). Lung inflation evokes a decrease in HNA (10).

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Recently, we have reported that pulmonary vagal C-fiber activation induces a visceral reflex, which results in a reduction in HNA (17). Hence, it appears that phasic discharge of the HN is modulated by a variety of sensory inputs. These inputs may act on some areas in the central nervous system and produce modulatory effects on the HN. Hence, direct activation of neurons or areas in the central nervous system may modulate inspiratory discharges of the HN.

Arginine vasopressin is a neurohormone synthesized in the hypothalamic paraventricular and supraoptic nuclei, and stored in the neurohypophysis. It is released into the peripheral circulation in response to dehydration or increase in plasma osmolality, and hemorrhage (27). Hence, the main function of AVP is to maintain water homeostasis by acting on the renal distal tubules and collecting ducts to increase water reabsorption. Deficiency of AVP due to a single gene mutation might lead to water diuresis, as exhibited in the Brattleboro rat (5). Additionally, AVP is also involved in the modulation of cardiovascular functions (18), and in other physiological functions, such as learning, memory consolidation, and retrieval (1). It also involves in social behavior (25), reproduction (23, 32), antipyretic effect (28), infections or stress (30), as well as exercise (14). Among these diverse biological actions, very little attention was paid to its modulation on respiratory function (35, 36). Our recent studies demonstrated that microinjection of AVP into the ventrolateral medulla (VLM) and/or rostral ventral respiratory group (rVRG) produced an inhibition on phrenic activity (3, 4). Because UAW patency and respiratory pump system must be regulated in a concerted manner, we therefore hypothesized that AVP-induced activation of neurons in the VLM and/or rVRG may also modulate the patency of the UAW. This prompted us to investigate if AVP-induced excitation of neurons in the VLM and/or rVRG may modulate phasic discharges of the HN to regulate the aperture of the UAW. Furthermore, we also examined if AVP-induced activation of AVP V_{1A} receptors on neurons within the VLM could produce an inhibition on phasic HNA. This will provide a notion that AVP may participate in the modulation of UAW aperture.

Materials and Methods

Animal Preparation

Nineteen male Wistar rats (278 ± 11 g) were used in the experiment. Eleven of these 19 animals were used for the dose-response of AVP injection into the medial and lateral ventrolateral medulla (VLM). Five animals were used for the study of AVP V_{1A} receptor and the remainder three were for phentolamine study. The rats were anesthetized with urethane (1.2 g/kg i.p.) subsequent to atropine pretreatment (0.5 mg/kg, i.m., Sigma, St. Louis, MO, USA). An additional dose of urethane (0.12 g/kg, i.v.) was given if necessary. The level of anesthesia was determined by observing a stable blood pressure (BP) and PNA when a nociceptive stimulus was applied to the paw. Catheterization of the femoral artery and vein was performed for BP monitoring and drug administrations, respectively. BP was monitored *via* a pressure transducer (Statham P23D, Grass Instrument, Quincy, MA, USA) connected to an amplifier (AC amplifier 7P1, Grass Instrument, Quincy, MA, USA) and recorded on a chart recorder (Grass, 7D) after calibration and was further digitized to be stored in hard disc by the PowerLab system (ADInstrument Pty Ltd. NSW, Australia). Tracheostomy was then performed. The vagus nerves on both sides were sectioned at the mid-cervical level. The rat was mounted in a stereotaxic instrument (Stoelting, Wood Dale, IL, USA) on a prone position, paralyzed with gallamine triethiodide (5 mg/kg, i.v., Sigma), and then artificially ventilated. The obex was exposed by removing part of the occipital bone and dura mater. End-tidal fractional concentration of CO_2 ($F_{ET_{CO_2}}$) was continuously monitored with a CO_2 analyzer (Electrochemistry CD3A, Pittsburgh, PA, USA) by sampling the gas from the tracheal tube *via* a 27 G needle and the rat was maintained at normocapnia in hyperoxia. Body temperature was maintained at 37°C with a heating pad.

Monitoring of Nerve Activities

After removing the shoulder on one side (usually the right side), the phrenic nerve (PN) was dissected by a dorso-lateral approach at the level of spinal C_4~C_5 and then cut peripherally. The central cut end of the PN was covered with saline-soaked cotton. During the experiment, the PN was carefully placed on a stainless steel bipolar electrode, which was connected to an amplifier (preamplifier P511, Grass, Quincy, MA, USA). Phrenic nerve activity (PNA) was amplified, filtered (0.3~3 kHz), integrated (R-C circuits, time constant = 0.5 sec) (10), and also displayed on an oscilloscope (Tektronix 5111A, Beavedon, OR, USA). Integrated PNA was recorded on the videotape via the PCM (Neuro Corda DR-890, New York, NY, USA) and/or simultaneously stored in the hard disc *via* the PowerLab system (ADInstrument).

The hypoglossal nerve (HN) was dissected *via* a ventral approach. After dissecting the digastric muscle, the HN could be identified clearly along the lingual artery and was carefully separated and then cut peripherally. During the experiment, the HN was placed on a stainless steel bipolar electrode. HNA was processed and amplified the same way as that of PNA, and was recorded either on the videotape or in the hard disc.

Microinjection Technique

Glass capillary (A-M system Inc, Everett, WA, USA) was heated and pulled with a puller (Narishige, Japan) into microelectrode, which was trimmed under the microscope to a tip of 20~30 μm in diameter (4). AVP was carefully infused into this microelectrode by a 32-gauge syringe. Drug was microinjected into the VLM by gas pressure generated by a pressure injector (Picosprizer IID, General Valve Corporation, Fairfield, NJ, USA). Glass microelectrode filled with drugs was fixed on a microelectrode holder (Stoelting), which was mounted on the stereotaxic apparatus and advanced to various regions of the VLM according to the coordinates of the rat brain atlas (24). The exact injection site was evaluated temporally by physiological responses to glutamate (Glu) injection and was confirmed by subsequent histological observation. The injected volume was adjusted to 15 and 30 nl, which was determined by repeated injections of a 10 ms interval. The exact volume microinjected was calculated by the displacement of fluid meniscus in the microelectrode, aided by the addition of a binocular microscope (Wild, Heerbrugg, Switzerland) with an ocular meter in one eyepiece.

Experimental Protocol

AVP (Sigma, St. Louis, MO, USA) was dissolved in saline (pH=7.4) to make a stock solution of 10 IU/ml, which was stored at -20°C. During experiment, this stock was diluted with saline (pH=7.4) containing 1% pontamine sky blue. Glu was also dissolved in saline to make a final concentration of 50 mM. AVP V_{1A}-receptor antagonist [β-mercapto-β,β cyclopentamethylenepropionyl^{1},-O-Me-Tyr^{2},Arg^{8}]-vasopressin (Not 11K13871, Sigma) was dissolved in saline to make a stock solution of 100 μg/ml and then stored at -20°C. This stock was diluted 10 times with saline during experiment.

According to our previous study (4), an effective area for the influence of AVP on respiration is located in the transitional region between the rostral VLM and the caudal VLM as identified by Glu. Two doses of AVP, 1.5×10^{-8} and 3.0×10^{-8} IU, were used to effectively induce a dose-dependent cardiopulmonary modulation as our recent reports (3, 4).

In the first experimental protocol, these two doses of AVP were randomly microinjected into the VLM and/or rVRG to produce a dose-dependent inhibition on PNA and phasic HNA at normocapnia in hyperoxia. There was an interval of 30 min between AVP administrations. The aim of this protocol was to examine whether AVP-induced activation of neurons in the VLM and/or rVRG produced a simultaneous modulation of PNA, HNA, and BP. The second protocol was designed to investigate whether reduction in HNA induced by AVP activation of neurons in the pressor area is baroreflex-dependent. Based on BP response in our recent reports (3, 4), the VLM could be divided into two subareas: medial and lateral. AVP-induced pressor effect could be only observed in the lateral but not in the medial VLM. We do not know whether AVP-induced modulation upon phasic HNA is caused by a baroreflex inhibition. In this protocol, only high dose of AVP was microinjected into the lateral VLM. The third protocol was designed to determine which subtype of AVP receptors might mediate cardiopulmonary modulation and UAW patency evoked by AVP administration. Hence, responses of PNA and HNA to AVP microinjection were evaluated before and after AVP V_{1A}-receptor antagonist administration into the same area. To accomplish this objective, pairs of glass microelectrodes, which were made by gluing two glass pipettes together, were used (3, 4). One of the microelectrodes was used for the injection of AVP and the other was used for the injection of AVP V_{1A} receptor antagonist.

Data Analysis and Statistical Evaluations

Data on the videotape were played back and digitized *via* the PowerLab system. These digitized data were first stored in the hard disc, then retrieved, and analyzed with software written by Visual C/C^{++}6.0. An average of twenty consecutive respiratory cycles before AVP administration was analyzed and averaged, and served as the control. Neuronal activities following AVP treatment were chosen as the experimental data and were further transformed into percent (%) of the control. T_I (duration of phrenic burst), T_E (period between phrenic burst), and T_{TOT} (sum of T_I + T_E) were also computed before and after AVP treatment. Mean BP before and after AVP injection were retrieved and analyzed by Data pad module of the PowerLab system. All data were expressed as mean ± S.E.M. (standard error of the mean).

Multiple comparisons were made to evaluate the data statistically. Thus, one-way ANOVA and then a modified *t*-test were performed to reject or accept the null hypothesis (37). Change in BP was evaluated by paired-*t* test. *P* values less than 0.05 are considered as significant between the control and experimental values.

Histological Verification

At the end of the experiment, the rat was sacrificed by infusion of overdosed urethane. The brain stem was carefully removed and stored in a 10% of formalin solution for one week. The brainstem tissue was serially sectioned at a thickness of 50 micron using a frozen microtome. The thin brain tissue slice was stained with Cresyl violet. Microinjection site of AVP

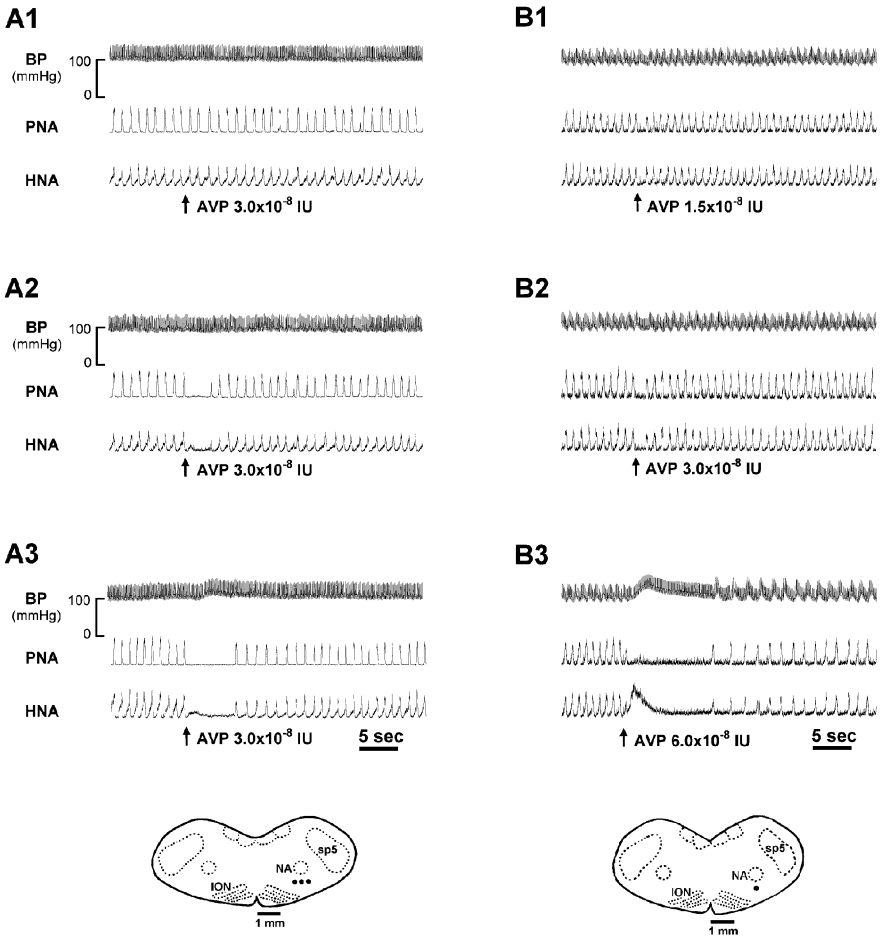

Fig. 1. Examples of the responses of phrenic nerve activity (PNA) and hypoglossal nerve discharge (HNA) to AVP microinjection into different subareas in the ventrolateral medulla (VLM), and/or the rostral ventral respiratory group (rVRG) which were observed. These subareas are located at sites with coordinates of 1.8 mm rostral to the obex, 1.8 mm ventral to the surface, and 1.6-2.0 mm to the midline with a medial-lateral distribution. AVP injected into the area producing only respiratory inhibition without changes in BP (panel A2), Type I response, was located at 1.8 mm lateral to the midline and the area inducing respiratory inhibition with a vasopressor effect (panel A3, Type II response, was founded in 2.0 mm lateral to the midline. AVP injection into an area more medial to 1.8 mm (panel A1) produced no changes in PNA, HNA and BP. Repeated injections of low- and high-dose of AVP into the area of Type I response evoked a reproducible inhibition on both PNA and HNA (panel B1 and B2). However, super high-dose of AVP (6.0×10^{-8} IU) administered by doubling the volume injected into the same area produced the Type II response (panel B3). The black dots in the brain section under each column represent the injection points. Panels A and B are taken from different rats. ION, the inferior olive nucleus; NA, the nucleus ambiguous; Sp5, spinal trigeminal tract.

was verified by comparison with the rat brain atlas (24). Data were excluded when the injection sites were verified to be outside the specific area of the VLM.

Results

Based on the change of BP following microinjection of AVP into the VLM, two types of response were obtained. In the Type I response, AVP-induced activation of neurons located in the medial portion of the VLM and/or rVRG produced only decreases in both PNA and HNA without changes in BP (Figs. 1A2, 1B1, 1B2, and 2B). In the Type II response, AVP-induced activation of neurons located in the lateral portion of the VLM and/or rVRG evoked not only decreased in both PNA and HNA but also increased in BP (Figs. 1A3 and 2B). Microinjection of AVP outside these two small areas did not produce any changes in PNA, HNA, and BP (Fig. 1A1). Saline injection into these small areas produced no effect on PNA, HNA and BP.

Decrease in PNA in Response to AVP-Induced Activation of Neurons in the VLM

Coordinates of the Type I area were 1.8 mm lateral to the midline, 1.6-1.8 mm rostral to the obex, and 1.8-2.4 mm ventral to the dorsal surface (Figs. 1 and 2), which was located between the pressor and depressor

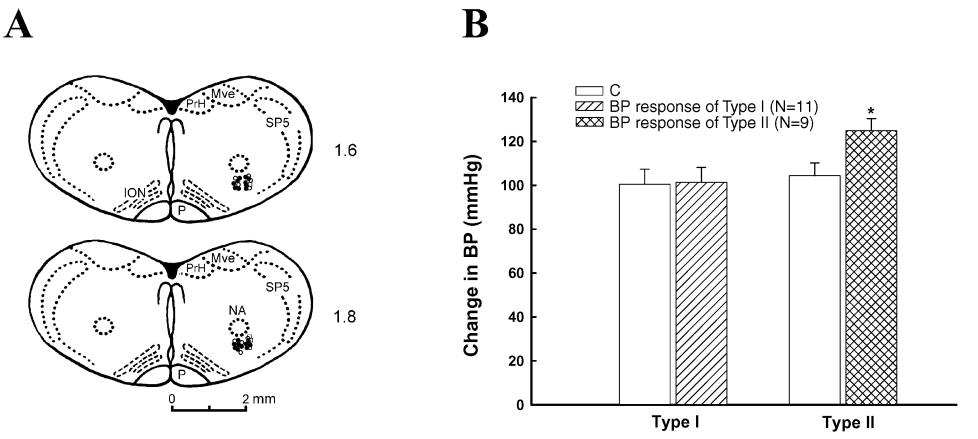

Fig. 2. Distribution of AVP-reactive sites in the medial and lateral VLM. The number of 1.6 and 1.8 in panel A represents the level rostral to the obex in mm. AVP microinjection into the medial and lateral subareas (● in panel A) produced no change in BP (Type I in panel B) and significant pressor effect (Type II in panel B), respectively. Points receiving AVP with its antagonist (□) and with phentolamine (○) were also given. C in panel B is the control. *$P < 0.05$ was the significant level when compared with the control by paired *t*-test. MVe, medial vestibular nucleus, PrH, nucleus prepositus hypoglossi. See other abbreviations in Fig. 1. N = number of rats observed.

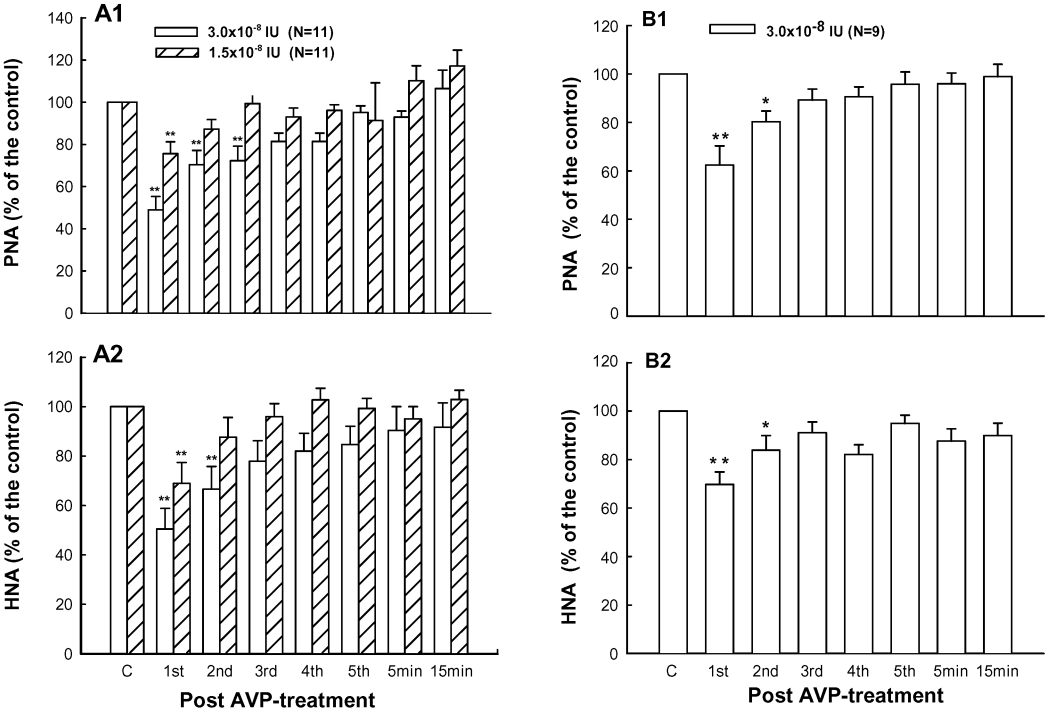

Fig. 3. Mean ± SEM (standard error of the mean) of PNA (panels A1 and B1) and HNA (panels A2 and B2) in Type I response (left panels) and Type II response (right panels) in response to AVP injection into the VLM and/or the rVRG. *$P < 0.05$; **$P < 0.01$ were the significant levels when compared with the control (C), which represents the consecutive twenty respiratory cycles before AVP administration, by multiple comparison test. N = number of rats observed.

areas of the VLM identified by Glu (5). Histological observation corresponded this small area to the rVRG.

Activation of neurons in the medial VLM and/or rVRG by AVP produced a dose-dependent decrease in PNA in eleven rats. With low-dose AVP activation (1.5×10^{-8} IU), PNA showed a short period of apnea followed by a decrease in amplitude, and then gradually returned to the control (Fig. 1B1). In grouped data, a significant decrease in PNA was displayed in the first neural breath after recovery from apnea, showing 76% of the control ($P < 0.01$, Fig. 3A1). High-dose AVP activation (3.0×10^{-8} IU) reduced PNA to 49%, 70%,

and 73% of the control in the first, second, and third neural breaths, respectively ($P < 0.01$, Fig. 3A1).

In the Type II response, AVP injected into the lateral VLM and/or rVRG, with coordinates of 2.0 mm lateral to the midline, 1.6-1.8 mm rostral to the obex, and 1.8-2.4 mm ventral to the dorsal surface (Figs. 1A3, 2A), also evoked apnea followed by a decrease in PNA (Fig. 1A3). Concurrently an increase in BP was observed (Fig. 2B). A significant decrease in PNA was observed in the first and second neural breaths from recovery of apnea, showing 62 % ($P < 0.01$, Fig. 3B1) and 80 % of the control ($P < 0.05$, Fig. 3B1), respectively.

Decrease in HNA in Response to AVP-Induced Activation of Neurons in the VLM

Response of phasic HNA to AVP activation was very similar to that of PNA (Figs. 1A and 1B). Hence, microinjection of low-dose AVP into the medial VLM and/or rVRG reduced HNA to 69% of the control in the first breath ($P < 0.01$, Fig. 3A2). High-dose of AVP treatment decreased HNA to 50% and 67% of the control in the first and second neural breaths, respectively ($P < 0.01$, Fig. 3A2). In the Type II response, high-dose AVP-induced excitation of neurons in the lateral VLM and/or rVRG lowered HNA to 70% and 84% of the control ($P < 0.05$, Fig. 3B2) in the first and second neural breaths, respectively. It is interesting to note that HNA was activated (unlike PNA) during the period of apnea in four of the 9 rats studied as shown in Figs. 1A3. However, this excitation to HNA caused by AVP injection during apnea was not quantified because there was no such an expiratory activity of the HN before AVP administration to transform the data as a percentage of the control.

Change in Respiratory Pattern Following AVP Activation of Neurons in the VLM

Activation of neurons in the medial and lateral VLM resulted in the prolongation of T_E (defined as apneic period or T_{EE}), which was evident immediately following AVP administration. Mean apneic period was 3.2 ± 0.5 sec and 5.2 ± 0.5 sec ($P < 0.05$) with low- and high-dose of AVP in the Type I response, respectively, and was 4.1 ± 0.1 sec ($P < 0.05$) in the Type II response. T_I and T_E after recovery from apnea tended to prolong but not significant.

Inhibition of PNA and HNA in the Type II Response

In three rats, inhibition of PNA and HNA in the Type II response was evaluated before and after phentolamine. AVP administration produced apnea and decreases in both PNA and HNA (Fig. 4A). Bolus

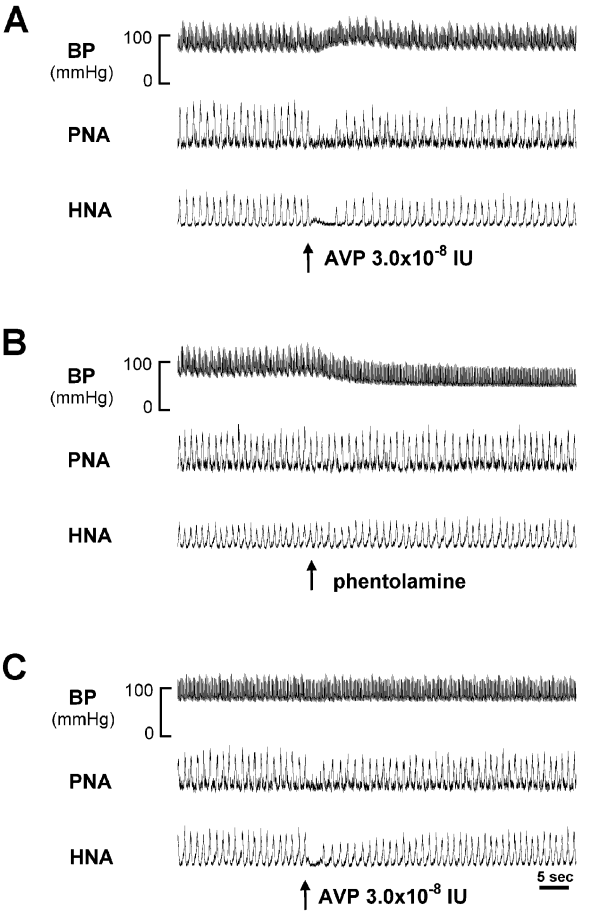

Fig. 4. Decreases in PNA and HNA in Type II response are not caused by baroreflex inhibition. Example was taken from an animal to show inhibition of PNA and HNA in combination with a vasopressor effect (panel A) in Type II response. Bolus injection of phentolamine (i.v.) reduced BP but did not affect the amplitude of PNA and HNA (panel B). After recovery of the BP, AVP injection into the same area still caused apnea and decrease in amplitude of PNA and HNA (panel C). See the injection point (□) in Fig. 2A and abbreviations in Fig. 1.

venous injection of phentolamine produced no changes in both of PNA and HNA (Fig. 4B) but hypotension. After administration of phentolamine, the same dose of AVP still produced an inhibitory modulation on both PNA and HNA, but no significant increase in BP (Fig. 4C).

Pressor Response Following AVP Activation of Neurons in the Lateral VLM

AVP-induced activation of neurons to produce pressor effect was only observed in the lateral but not in the medial VLM. This lack of a change in BP was used as a criterion to classify responses into the Type I and the Type II in the present study. Increases in BP

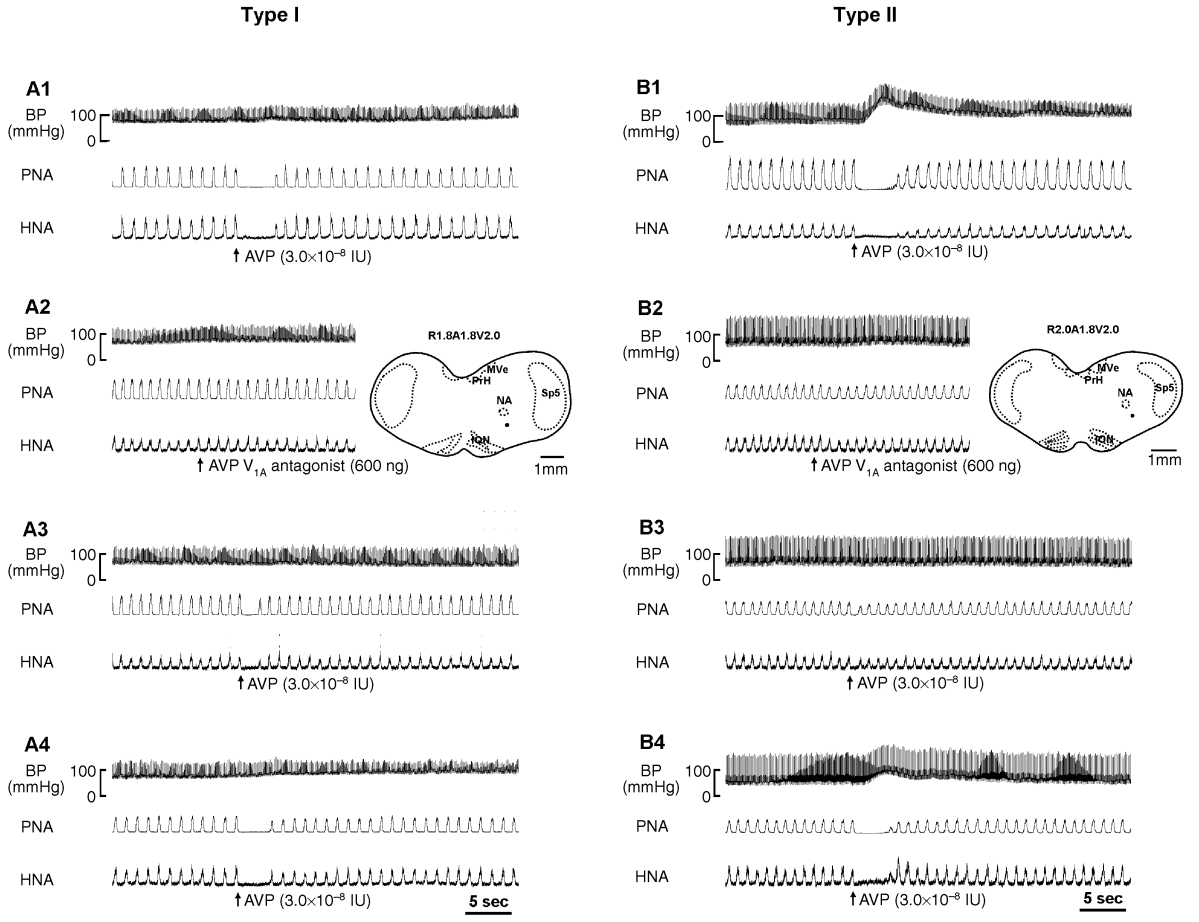

Fig. 5. Cardiopulmonary responses to AVP-induced neuronal activation are mediated via AVP V_{1A} receptors on neurons located in the VLM and/or the rVRG. AVP-induced inhibition of PNA and HNA was observed in both Types I and II responses (panels A1 and B1), and vasopressor effect was only seen in Type II response (panel B1). Microinjection of AVP V_{1A} receptor antagonist into the same areas as microinjection of AVP (panels A1 and B1) did not produce any changes in cardiopulmonary functions (panels A2 and B2). Injection of AVP following the administration of antagonist did not produce cardiopulmonary effects (panels A3 and B3), demonstrating that the antagonist effectively abolished cardiopulmonary changes induced by AVP. This antagonistic effect was reversible so that the effect of AVP was regained when AVP was injected for 30 min after the antagonist (panels A4 and B4). The black dot in the brain section on the right side of each panel represents the injection points. See Figure 1 for abbreviations.

ranged from 15 to 30 mmHg. Mean BP was 104 mmHg before AVP treatment and rose to 125 mmHg after AVP treatment ($P < 0.01$, Fig. 2).

Cardiopulmonary Modulation Mediated through AVP V_{1A} Receptors

Microinjection of AVP V_{1A} receptor antagonist did not evoke changes in PNA, HNA (Fig. 5A2 and 5B2), and BP (Fig. 5B2). However, it abolished the modulation of AVP on PNA, HNA, and BP. Hence, AVP-induced decreases in PNA and HNA seen in the Type I and II responses (Figs. 5A1 and 5B1), as well as pressor response seen in the Type II disappeared after pretreatment with the AVP V_{1A} receptor antagonist (Figs. 5A3 and 5B3). This antagonistic effect was reversible; control levels of PNA, HNA, and BP reappeared 30 minutes after the pretreatment of the antagonist (Figs. 5A4 and 5B4).

Mean PNA was reduced to 70 % of the control in the Type I ($P < 0.01$, Fig. 6A1) and 67% of the control in the Type II responses ($P < 0.05$, Fig. 6B1). After pretreatment of low- and high-dose of the AVP antagonist, microinjection of AVP produced a decrease in PNA to 89% and 95% of the control ($P > 0.05$, Figs. 6A1 and 6B1). Thirty minutes later, AVP injection was lowered PNA to 67 % of the control ($P < 0.01$, Fig. 6A1) in the Type I and to 57 % of the control in the Type II responses ($P < 0.01$, Fig. 6B1).

Mean HNA was decreased to 76 % of the control in the Type I ($P < 0.01$, Fig. 6A2) and to 62% of the control in the Type II response ($P < 0.015$, Fig. 6B2).

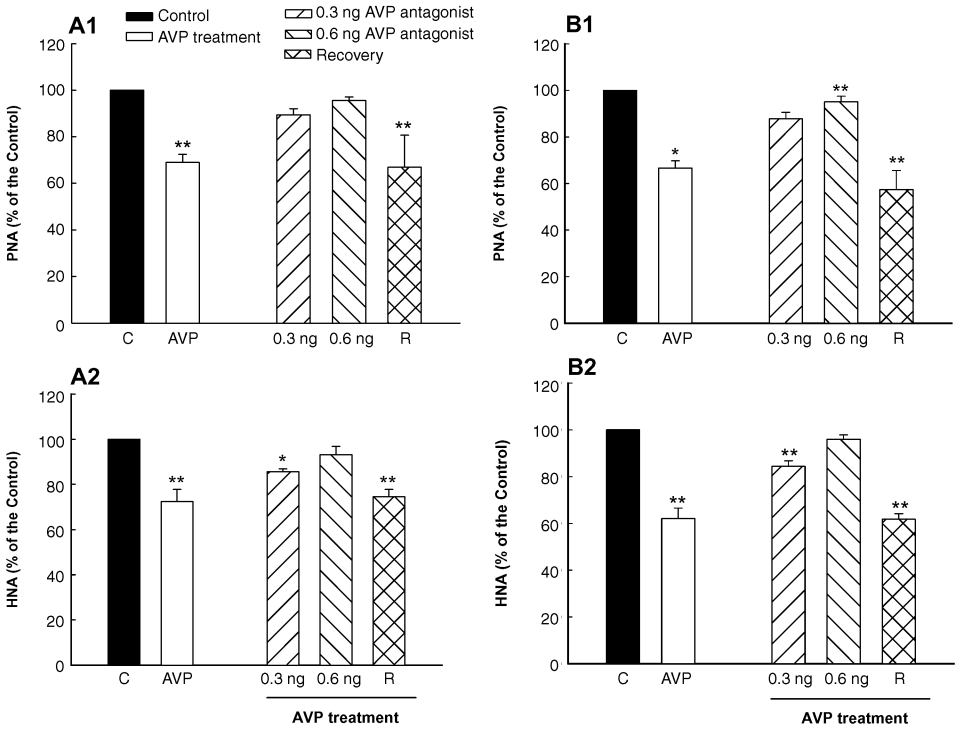

Fig. 6. Mean values ± SEM of PNA and HNA before and after administration of AVP V_{1A} receptor antagonist. AVP-induced activation of neurons in the VLM and/or rVRG produced significant decreases in both PHA and HNA (white columns) compared with the control (C, black columns), before AVP V_{1A} receptors antagonist administration regardless of Type I or II response. These reductions were partially attenuated by low-dose of and totally abolished by high-dose of AVP V_{1A} receptor antagonist (striped columns). The reversibility with antagonist attenuation was demonstrated by the return of substantial reductions of nerve amplitude induced by AVP 30 min after the administration of antagonist (crossed columns). $*P < 0.05$; $**P < 0.01$ were the significant levels when compared with the control by multiple comparisons test. Five rats were observed.

Low-dose AVP V_{1A} receptor antagonist did not abolish the inhibition of AVP-induced activation of neurons in the VLM and/or rVRG on HNA, which was 86% of the control in the Type I response ($P < 0.05$, Fig. 6A2) and 84 % of the control in the Type II responses ($P < 0.01$, Fig. 6B2), respectively. High-dose AVP V_{1A} receptor antagonist totally abolished the inhibitory modulation of AVP on HNA in both the Type I and Type II responses ($P > 0.05$, Figs. 6A2 and 6B2). Thirty minutes after V_{1A} receptor antagonist, AVP-induced activation of neurons in the VLM and/or rVRG reduced again HNA to 75% and 62% of the control in the Type I and Type II responses ($P < 0.01$, Figs. 6A2 and 6B2), respectively. These results showed that the observed antagonistic effect of the AVP V_{1A} receptor antagonist is reversible.

The AVP V_{1A} receptor antagonist also attenuated the prolongation of apneic period. For convenience, we transformed the apneic period into % of the control, a ratio of apneic period/T_E. Hence, this ratio was 4.4% and 8.5% of the control in the Type I and the Type II responses, respectively ($P < 0.01$, Figs. 7A and 7B), and was totally abolished by the V_{1A} receptor antagonist ($P > 0.05$, Figs. 7A and 7B). Significant prolongation of apnea reappeared 30 minutes following the administration of the antagonist ($P < 0.01$, Figs. 7A and 7B).

Discussion

There are three main findings in this study. First, microinjection of AVP into the medial and lateral regions of the VLM and/or rVRG produced an inhibition on phasic HNA. Second, this inhibition was not caused by baroreflex. Third, this inhibition was mediated through V_{1A} receptors, which were located in the neurons within the VLM and/or rVRG. Our present findings provided the first evidence that neurons, with AVP V_{1A} receptors and located in the VLM and/or rVRG, may participate in the regulation of UAW patency by modulating HNA.

Microinjection of AVP

We have recently reported that microinjection of a volume of AVP less than 30 nl into the VLM and/

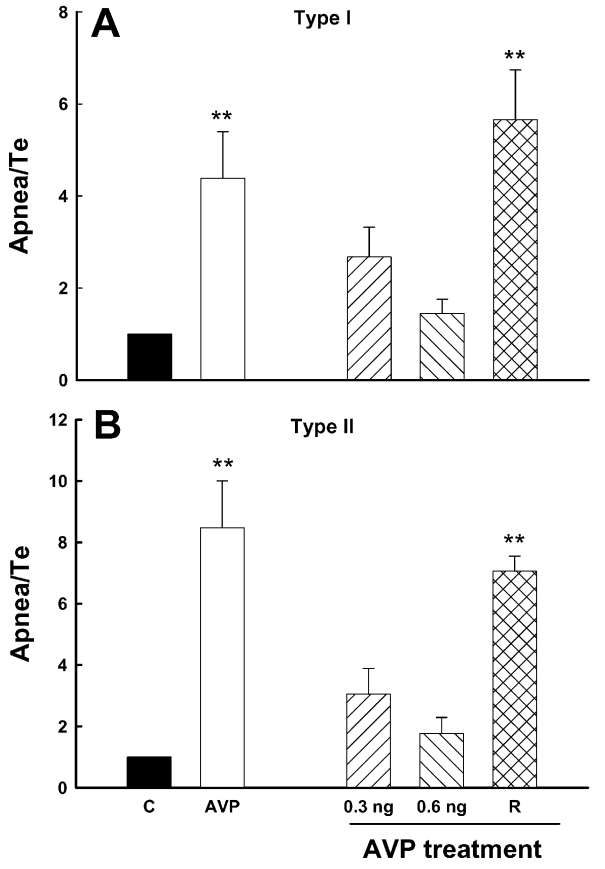

Fig. 7. Mean values ± SEM of apneic period before and after AVP V_{1A} receptors antagonist administration. AVP-induced activation of neurons in the VLM and/or rVRG produced a significant prolongation of expiration (white columns), compared with the control (C, black columns) before AVP V_{1A} receptors antagonist administration regardless of Type I (panel A) or Type II (panel B) response. These prolongations were totally abolished by low- and high-dose of AVP V_{1A} receptor antagonist (striped columns). These attenuations by antagonist disappeared such that a significant prolongation of expiration induced by AVP was again observed 30 min after the administration of antagonist (crossed columns). *$P < 0.05$; **$P < 0.01$ were the significant levels when compared with the control by multiple comparisons test. Five rats were observed.

or rVRG is suitable for the investigation of the effect of AVP on respiration (3, 4). With this volume of injection, AVP excites neurons within the VLM and/or rVRG and produces inhibitory modulation on respiration. Unlike Glu, this excitatory action of AVP on neurons probably did not evoke neuron toxicity or depolarization blockade since similar responses were observed following repeated injections of AVP. However, an injection volume larger than 30 nl may cause diffusion of injected materials out of the injection area. This phenomenon might explain why large volume of AVP injected into the medial region (Type I area) still produced a Type II response (Fig. 1B3). In other words, an injection volume larger than 30 nl may also excite surrounding neurons located in a distance of 200 μm or more away if the drug diffused evenly from the injection site. Thus, neurons in the medial and the lateral VLM and/or rVRG, which are located in a medial-lateral direction, may play a differential modulation of cardiopulmonary functions. In addition they may also be involved in the modulation of a patent UAW. The diffusion problem of microinjection was repeatedly observed in other studies (3, 4, 21).

Decrease in Phasic HNA Caused by AVP

Respiratory-related activity of the HN and/or GG generates the dilating force to counteract the constricting force for the UAW generated from the negative intrapleural pressure during inspiration. A balance between these two forces is important to maintain a patent UAW. Otherwise, the dilating force will be overridden by the constricting force and may result in a decrease in the airway diameter or even collapse of the UAW, as has been observed in the patient with obstructive sleep apnea syndrome (8, 26). This phenomenon has also been demonstrated in animal study (2). Our present data revealed that a significant decrease in HNA was discerned as neurons in the VLM and/or rVRG were excited by AVP. These results indicated that neurons in the medial and lateral VLM and/or rVRG may be involved in the modulation of a patent UAW.

The HN consists of two branches: the medial and the lateral, which innervate the GG and styloglossus, respectively. Recently, it has been reported that co-activation of these two branches may play an essential role in the development of a stiff but patent UAW (6, 7). Activities of the HN recorded in the present study must represent the whole nerve containing both branches. Decreases in HNA with AVP microinjection may associate with a decrease in diameter and anteroposterior space of the oropharynx. This situation might give the UAW to be susceptibly insulted by other factors.

AVP-Induced Inhibition of HNA

Based on our present data, it appears that neurons within the VLM appeated to contain AVP V_{1A} receptors. These neurons activated by AVP may participate in the modulation of UAW patency by the inhibition on HNA. The low-dose of AVP antagonist could not abolish all of the modulatory effects induced by AVP. This may be due to low dose of AVP antagonist saturates part of the AVP receptors in the

area. It may also be explained by the fact that the injection area of AVP and its antagonist may not be identical because of the differences in molecular weight of the drug, spatial variation between the two parallel microelectrodes, and disparity of the tissue resistance. Doubling the injection volume of the antagonist would increase the dose effect, which might abolish totally the action of AVP. Besides, AVP antagonist alone did not produce any significant influences on HNA and PNA. Hence, intrinsic AVP receptors in these areas may play a modulatory role on UAW patency and pulmonary functions.

Neurons with V_{1A} receptors may be distributed in a medial-lateral direction. Neurons involved in the modulation of the respiratory system are mainly distributed on the medial portion, while those involved in the modulation of the cardiovascular system are probably located in a more lateral area. This uneven neuronal distribution would explain why the Type I response was obtained from the medial VLM and/or rVRG, whereas the Type II response was observed in the lateral area.

Our data suggested that AVP, through its action on V_{1A} receptor presenting on neurons within the VLM and/or rVRG, were involved in the central modulatory mechanism of respiration and UAW aperture. Very little attention has been paid to this field (3, 4, 15). More research is needed to elucidate the questions regarding the involvement of AVP in the central modulation of respiration.

Our data also suggested that an intrinsic vasopressinergic pathway may project to the VLM and/or rVRG. However, at present the origin of this putative pathway is unclear. The origin of this pathway is most likely the paraventricular nucleus of the hypothalamus. This putative pathway has been inferred from physiological and histological observations (22, 31). Based on our present and recent observations (3, 4), this pathway seemed to be comprised of two branches: one projecting to the medial portion and the other innervating neurons in the lateral VLM. Yet, our data did not reveal whether these neurons with V_{1A} receptors were interneurons or premotor neurons.

Physiological Considerations

AVP is mainly involved in water balance. It is released primarily in response to an increase in plasma osmolality or a loss of blood volume. Intuitively, it is reasonable to question whether respiration has to be modulated whenever there is a change in plasma osmolality resulting in AVP release (3). This "paradox" may be explained as follows: First, high osmolality may not stimulate the pathway responsible for AVP-induced changes in cardiopulmonary functions, and/or the levels of AVP released with high osmolality may not be high enough to produce cardiopulmonary modulations. Second, it has been reported that infection/stress may induce release of AVP (20). In chronic infection, corticotropin-releasing hormone (CRH) is negatively inhibited by the increase of plasma corticosteroid. Under repeated or chronic stress, a shift from the CRH-dependent response to an AVP-predominant pattern may gradually develop (16). Third, high-intensity exercise is generally considered as stress. During exercise, AVP has been demonstrated to play a role in modulating cardiovascular functions, e.g., pressor effect and blood redistribution (14, 33). However, during high-intensity exercise with the maximum oxygen consumption above 70%, AVP is largely released to replace the deficiency in CRH secretion and to maintain the function of the hypothalamic-pituitary-adrenal axis (14). Unfortunately, this involvement of AVP in modulating cardiopulmonary functions during heavy exercise seemed to be unfavorable because it inhibits respiration and increases UAW resistance. On the other hand, AVP may provide a protective mechanism to prevent one from "over-exercising" by inhibiting respiration, thus reducing the supply of oxygen. More research is needed to further elucidate the involvement of AVP in the modulation of cardiopulmonary functions during exercise.

In conclusion, the present study showed that AVP-activated neurons within the VLM and/or rVRG could modulate the coordination of UAW aperture and respiratory pumping system in concomitant with cardiovascular functions. This AVP-mediated modulation on cardiopulmonary functions may play an essential role in some physiological situations, such as stress and exercise. More research is necessary to clarify the role of AVP in the modulation of cardiopulmonary functions in varied physiological situations.

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