Cardiopulmonary Response to Vasopressin-Induced Activation on V_{1A} Receptors in the Lateral Ventrolateral Medulla in the Rat

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Abstract

The aim of the study was to examine whether or not arginine vasopressin (AVP) might modulate cardiopulmonary functions by acting on the lateral area of the ventrolateral medulla (VLM) in the rat. The rat was anesthetized, bilaterally vagotomized, paralyzed, ventilated, and then placed on a stereotaxic instrument in a prone position. Activity of the phrenic nerve (PNA) was monitored at normocapnia and hypercapnia in hyperoxia. Microinjection of AVP into the lateral region of the VLM resulted in a brief apnea followed by a significant decrease in PNA amplitude and a concomitant significant increase in blood pressure. The inhibition of PNA with AVP treatment could be partly attenuated by hypercapnia but not by phentolamine. Both inhibition of PNA and pressor response with AVP microinjection into the lateral VLM were totally abolished after pretreatment with AVP V_{1A} receptor antagonist. These results suggest that a vasopressinergic pathway projects to the lateral VLM and modulates cardiopulmonary functions *via* AVP V_{1A} receptors on neurons within the lateral VLM.

Key Words: arginine vasopressin, AVP V_{1A}-receptor antagonist, ventrolateral medulla, phrenic nerve activity, hypercapnia, blood pressure, rat

Introduction

Arginine vasopressin (AVP) is synthesized mainly in the paraventricular and supraoptic nuclei in the hypothalamus and stored in the neurohypophysis. It is released into the circulation in response to an increase in plasma osmolality or a decrease in blood volume (30, 39). Circulating AVP acts on the renal distal convoluted tubules and collecting ducts to increase water reabsorption and maintain water homeostasis during dehydration and/or hemorrhage. Accordingly, AVP deficiency would lead to water diuresis, a condition clearly demonstrated in the Brattleboro rat which, because of a single gene mutation, cannot synthesize AVP (8, 34). Arginine vasopressin was so named for its powerful vasoconstricting effect when given intravenously (16, 39). Recently, AVP was found to exhibit diverse biological effects (28) such as memory and learning mechanisms (1), as well as social and reproductive behaviors (26, 36). It displays a preovulatory surge during the reproductive cycle in the rat (24). Additionally, AVP has also been demonstrated to have antipyretic activities (31) and maintain the responsiveness of hypothalamic-pituitary adrenal axis to stress (27, 32). Despite these diverse biological actions of AVP, there is little information regarding its modulatory activities in respiration. We have previously demonstrated that peripheral administration of AVP resulted in an increase in airway resistance, a reduction in lung compliance, and a concomitant pressor effect (11). Furthermore, Walker and Jennings observed that excitatory effects of angiotensin II on respiration was substantially augmented by the pretreatment of AVP V1-receptor blockade in the conscious dogs but not in rats (40, 41, 42, 43). These results suggest that AVP might have an inhibitory effect

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on respiration. Our recent study showed that microinjection of AVP into a specific area of the ventrolateral medulla (VLM) could produce apnea followed by a reduction in phrenic discharge without pressor effect. This modulatory action of AVP is mediated *via* V_{1A}-receptors found on neurons within the VLM (5).

The VLM is a longitudinal column extending from the posterior edge of the facial nucleus to the level of the first spinal cord (4, 18, 29). Part of the VLM constitutes the main body of the ventral respiratory group (VRG), which is located underneath the nucleus ambiguus (NA) and nucleus retroambigualis (NRA) in the rat. The VRG plays an important role in respiratory regulation in the rat. The VLM can be divided into two areas: a pressor area located in the rostral ventrolateral medulla (rVLM) and a depressor area in the caudal VLM (cVLM) (5, 13, 46). A question initiated by our recent results is whether or not AVP could evoke an inhibitory effect on respiration with a concomitant pressor effect by acting on the pressor area of the VLM. To answer this question, AVP and its antagonist were microinjected into the same area of the VLM through pairs of microelectrodes. The results of the present study clearly demonstrate that AVP does exhibit an inhibitory effect on respiration as well as a pressor effect by acting on V_{1A} receptors located in neurons within the lateral area of the VLM.

Materials and Methods

Animal Preparation

Male Wistar rats (330 ± 16 g) were used. The rat was anesthetized with urethane (1.2 g/kg i.p.) following atropine pretreatment (0.5 mg/kg, i.m., Sigma, St. Louis, USA). The level of anesthesia was evaluated by a stable recording of blood pressure (BP) and phrenic nerve activity (PNA). Supplementary dose of urethane (0.12 g/kg, i.v.) was given when changes in BP and PNA were seen in response to nociceptive stimuli applying to the paw. The femoral artery and vein were catheterized for monitoring BP and administration of drugs, respectively. BP was monitored *via* a pressure transducer (Statham P23D, Grass Instrument, Quincy, MA, USA) and was amplified (AC amplifier 7P1, Grass Instrument, Quincy, MA, USA). After calibration, signal of BP was recorded on a chart recorder (Grass, 7D) and further digitized to be stored in hard disc by the PowerLab system (ADInstrument Pty Ltd. NSW, Australia). Tracheostomy and bilateral cervical vagotomy were performed. The rat was then mounted on a prone position in a stereotaxic instrument (Stoelting, Wood Dale, Illinois, USA), paralyzed with gallamine triethiodide (5 mg/kg, i.v., Sigma), and ventilated artificially. 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) and maintained at normocapnia in hyperoxia. Its body temperature was maintained at 37-38°C with a heating pad or lamp.

Recording of Phrenic Nerve Activity

The phrenic nerve (PN) was dissected by a dorso-lateral approach at the level of spinal C_4~C_5 and cut peripherally. The central cut end of the PN was placed upon a stainless bipolar electrode. Rough PNA was amplified (preamplifier P511, Grass Instrument, Quincy, MA, USA), filtered (0.3~3 kHz), integrated (R-C circuits, time constant = 0.5 sec) (12), and then displayed on an oscilloscope (Tektronix 5111A, Beavedon, Oregon, USA). Integrated PNA was recorded onto a videotape *via* PCM (Neuro-Corda 890, New York, NY, USA) and/or simultaneously stored in hard disc *via* the PowerLab system (AD Instrument).

Microinjection Technique

Glass pipette was heated and pulled with a puller into microelectrode, which was trimmed under the microscope to a tip of 20~30 μm in diameter. AVP was carefully infused into this glass microelectrode by a 32-gauge syringe. Drug was microinjected into the brain tissue by gas pressure, generated by a pressure injector (Picosprizer IID, General Valve Corporation, Fairfield, NJ, USA). Glass microelectrode filled with drug was fixed on a microelectrode holder (Stoelting), which was mounted on the stereotaxic instrument and advanced to various regions of the VLM according to the coordinates of the rat brain atlas (25) and confirmed by physiological responses to glutamate (Glu) injection. The volume of microinjection 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

Arginine vasopressin (Sigma) was dissolved in saline (pH = 7.4) to make a solution of 10 IU/ml and stored at -20°C as stock. This stock was then diluted with saline (pH = 7.4) containing 1% pontamine sky blue during experiment. Glu was dissolved in saline, which contained dye 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 (Sigma, St. Louis, MO, USA) was dissolved in saline to make a solution of 100 μg/ml and stored at -20°C as stock. This stock was diluted 10 times with saline during the day of experiment.

To evaluate an effective location or area for the study of the influence of AVP on respiration, a pilot study was conducted to identify the rVLM from the cVLM by Glu microinjection into various regions of the VLM (5). In the pilot study, the rostral VLM (rVLM) was distinguished from the caudal VLM (cVLM) based on the pressor and depressor effects, respectively.

Four experimental protocols were performed. In the first protocol, two doses of AVP, 1.5×10^{-8} and 3.0×10^{-8} IU, which have been demonstrated to be effective in producing pulmonary responses (5), were used. These two doses of AVP were randomly microinjected into the lateral area of the VLM at normocapnia in hyperoxia. An interval of 30 minutes between AVP injections was taken. The purpose of this protocol was to examine whether AVP could produce a simultaneous inhibitory effect on respiration and a pressor effect. In the second protocol, this inhibitory action of AVP was then evaluated at a high level of $F_{ET}CO_2$. The aim of the second protocol was to examine if this inhibitory action could be attenuated by high concentration of CO_2. Thus, responses of BP and PNA to AVP microinjection were evaluated before and after increasing $F_{ET}CO_2$ to a level of 0.08. The third protocol was performed to examine whether or not the inhibitory effect of AVP on respiration is caused by a baroreflex. Hence, cardiopulmonary responses to microinjection of AVP were assessed before and after phentolamine mesylate (50 μg/100 g, RBI, MA, USA). The fourth protocol was designed to determine the type of AVP receptors that might mediate this inhibitory effect of AVP on cardiopulmonary functions. Thus, cardiopulmonary responses to AVP treatment were evaluated before and after AVP V_{1A}-receptor antagonist administration into the same area. Pairs of glass microelectrodes were made and trimmed in the same way as that in our recent report (5). One of the microelectrodes was used for microinjection of AVP and the other for AVP antagonist.

Data and Statistical Analysis

The data on the videotape were digitized *via* the PowerLab system, and the data in the hard disc were retrieved and then analyzed by software. An average of ten consecutive respiratory cycles before AVP treatment was used as the controls. Neural activities following AVP treatment was chosen as the experimental data and were further transformed into percent (%) of the controls. Mean BP before and after AVP treatment were retrieved and analyzed by Data pad module of the PowerLab system. All the data were expressed as mean ± S.E.M. (standard error of the mean).

T_I (period for phrenic inspiration), T_E (period between phrenic inspiration) and T_{TOT} (sum of T_I + T_E) were also computed before and after AVP treatment. T_{EE} represents the extension of T_E during the period of cessation of PNA or apnea caused by AVP treatment.

These data were compared in multiple aspects (44). One-way or two-way ANOVA was executed and then a Dunnett's modified *t*-test (6) was performed. P value smaller than 0.05 was considered as statistically significant.

Histological Verification

The rat was sacrificed by an overdose of anesthetics after completion of the experiment. The brain stem was quickly removed and stored in a 10% of formalin solution for at least one week. A series of transverse sections of the brainstem were cut at 50 micron using a frozen microtome. The thin brain tissue slice was stained with Cresyl violet. The microinjection site of AVP was verified by a comparison with the rat brain atlas (25). When microinjections were administered outside the specific area of the lateral VLM, the data were excluded.

Results

The effects of AVP in various areas of the VLM, dorsal medulla, and medullary raphe nucleus on cardiopulmonary functions were examined. Cardiopulmonary responses were only observed when AVP was microinjected into the lateral region of the VLM.

Differentiation of VLM into Pressor and Depressor Areas by Glu

The areas explored in the present study are located in a longitudinal region with a coordinate of 1.8-2.0 mm lateral to the midline, 1.4-2.2 mm rostral to the obex, and 1.8-2.4 mm ventral from the surface of the brain stem. Pressor area identified by Glu was located in 2.2-2.0 mm rostral to the obex, and depressor area was located 1.4 mm rostral to the obex (Fig. 1). A transient area between the rVLM and cVLM located around 1.8-1.6 mm rostral to the obex produced no prominent changes in BP with Glu (Figs. 1C and 1D). In all these regions, Glu-activated neurons evoked apnea followed by a reduction in PNA. A seizure-like activity of PNA, showing several bursts with low amplitude and high frequency (Fig. 1C), was observed in some animals as Glu was injected into the area

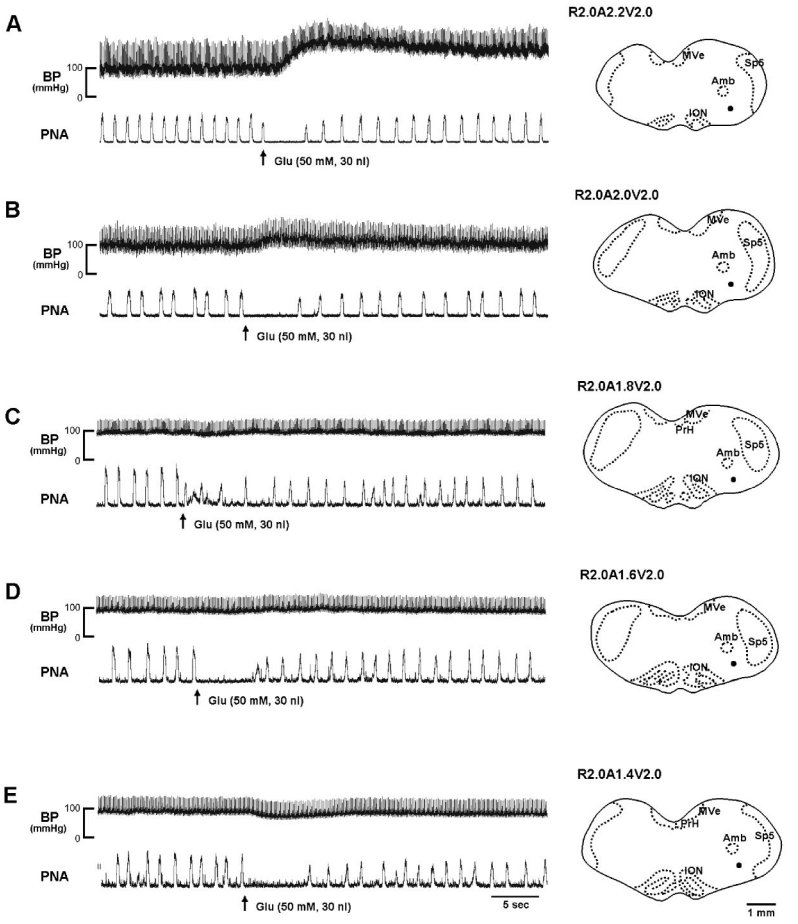

Fig. 1 Cardiopulmonary responses to glutamate (Glu) excitation to the ventrolateral medulla (VLM) were shown in one of the animal studied. Based upon the changes in blood pressure (BP) caused by Glu stimulation to the neurons within the VLM, a pressor area and a depressor can be distinguished. The pressor area was localized in the rostral portion of the VLM (rVLM) with a distance of about 2.0 mm anterior to the obex and produced increases in BP (A and B) in response to Glu (upward arrow). In contrast, the depressor area was located 1.4 mm anterior to the obex (cVLM). Excitation of the cVLM caused a decrease in BP (panel E). Simultaneously, an inhibition of phrenic nerve activity (PNA) was displayed when neurons within the VLM were excited. There is a transient area between rVLM and cVLM (panels C and D). Note that PNA exhibited either excitation or inhibition following Glu administration in this transient area. Thus, the VLM constitutes a longitudinal strip with a rostrocaudal direction. Black spot on each brain section represents the injection site. R: right to the midline; A: rostral to the obex; V; below the surface of the obex; ION, the inferior olive nucleus; Amb, the nucleus ambiguus, Mve, medial vestibular nucleus, PrH, nucleus prepositus hypoglossi, Sp5, spinal trigeminal tract.

around 1.8 mm rostral to the obex.

Effect of AVP-Induced Activation of Lateral VLM on PNA

Microinjection of AVP into the lateral VLM, which was located in 1.6-2.2 mm rostral to the obex, 2.0 mm lateral from the midline, and 1.8-2.4 mm ventral from the brain surface, produced apnea followed by a decrease in amplitude of PNA, and a concomitant pressor effect (Fig. 2). The major effective area was around 1.6-1.8 mm rostral to the obex (Figs. 2C, 2D, and 3). This coordination corresponds to the Glu-identified transient area and is 0.2 mm more lateral to an area of our recent observation (5), showing an inhibition of respiration without causing any change in BP. This area is the latearal VLM.

A histological examination showed that a spot of 200 microns in diameter was observed in a single injection site. Traces of dye were not observed along the microelectrode track, which suggests that AVP should only affect neurons around the injection site. No cardiopulmonary responses were observed following injection of an equal volume of saline (pH = 7.4) (Fig. 2G) indicating that changes in PNA and BP were caused by AVP injection, rather than a mechanical volume expansion. Injection of AVP into sites beyond the lateral VLM, such as the dorsal medulla (DM) (data not shown) and midline medullary raphe nucleus (MRN), did not result in changes in PNA and BP (Fig. 2F). The DM and MRN had been demonstrated to

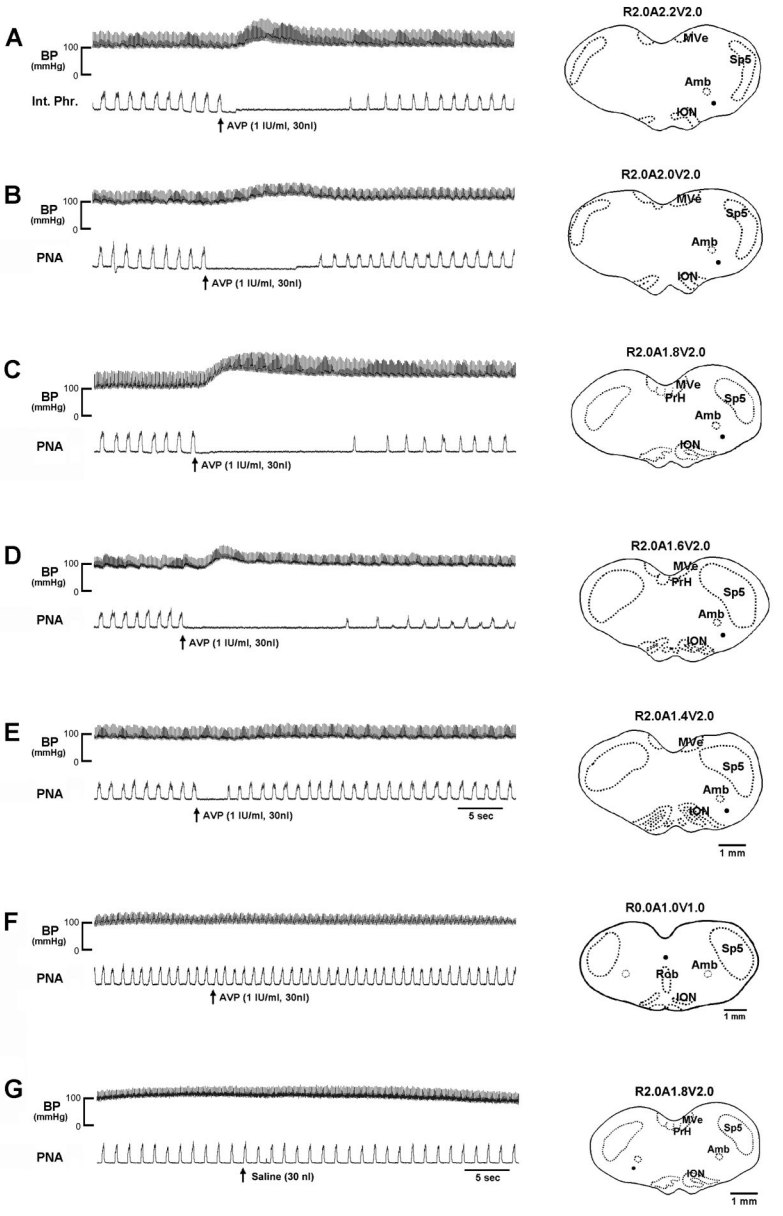

Fig. 2 Responses of PNA and BP to microinjection of arginine vasopressin (AVP) into the areas of the VLM were traced. Apnea followed by a reduction in PNA and a pressor effect were observed in panels A, B, C, D, and E, which represent different subareas of the VLM from 1.4-2.2 mm rostral to the obex, and 2.0 mm from the midline and 2.0 mm beneath the brain surface. Microinjection of AVP into the midline raphe nucleus produced no changes in PNA and BP (panel F). Saline vehicle microinjection into the VLM did not evoke cardiopulmonary responses (panel G). The data in panels F and G were obtained from different animals. The upward arrowhead on each panel indicates AVP microinjection with a concentration of 3.0×10^{-8} IU and an injection volume of 30 nl. Black spot in the diagrams on the right side of each panel is the injection site. See Fig. 1 for abbreviations.

modulate cardiopulmonary functions (10, 13). So, we believed that a specific area between the rVLM and the cVLM must contain receptors or binding sites for AVP.

The data collected from fifteen animals (Fig. 3) were grouped and averaged. Low doses of AVP (1.5×10^{-8} IU) produced reductions of PNA to 78.19% (Fig. 4, $P < 0.01$) and 88.8% (Fig. 4, $P < 0.05$) of the control in the first and second breath after AVP treatment, respectively. High doses of AVP (3.0×10^{-8} IU) evoked significant decreases in PNA from the first to 4th neural breath (Fig. 4, $P < 0.01$). Thus, low doses of AVP produced a smaller reduction in PNA than those produced by high doses (Fig. 4).

Alteration of Respiratory Pattern with AVP Treatment

AVP microinjection into the lateral VLM

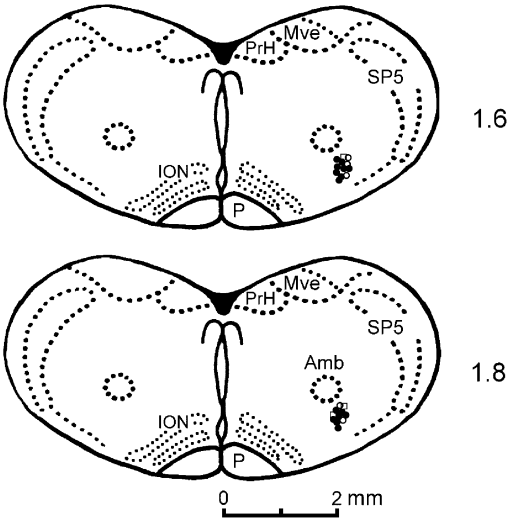

Fig. 3 Distribution of AVP-reactive sites in the lateral VLM. The level was from 1.6 to 1.8 mm anterior to the obex. Points receiving microinjection of AVP alone (●), AVP with its antagonist (○), and AVP with phentolamine (□) were marked. See Fig. 1 for abbreviations.

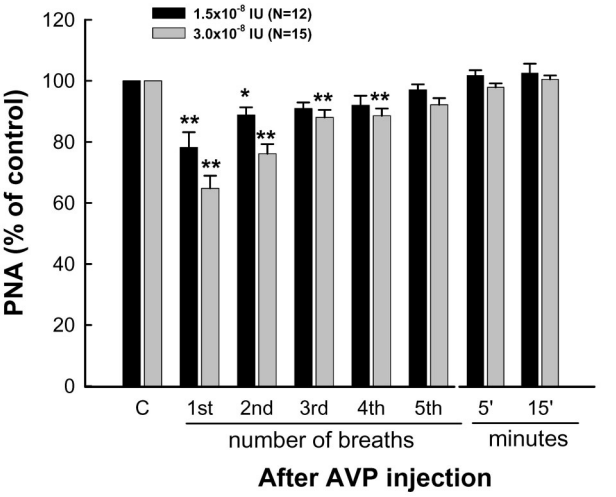

Fig. 4 PNA (expressed as % of the control) responds to AVP microinjection into the lateral VLM. Bars represent mean ± standard error of the mean (S.E.M). C is the control, which represents an average of the consecutive ten neural respiratory cycles before AVP microinjection. N is observation numbers. *$P < 0.05$; **$P < 0.01$, compared with C.

provoked significant changes in T_E but not in T_I. Low doses of AVP prolonged T_E from 0.93 ± 0.12 s before AVP treatment to 3.13 ± 0.30 s (Fig. 5A, $P < 0.01$) for the extension of T_E (T_{EE}) during apnea, and to 1.07 ± 0.11 s (Fig. 5A, $P < 0.01$) for the first respiratory cycle following recovery from apnea. High doses of AVP initiated a prolongation of T_E from 0.93 ± 0.07 s to 6.18 ± 1.03 for T_{EE}, and to 1.19 ± 0.09 ($P < 0.01$, Fig. 5B) and 1.06 ± 0.08 s ($P < 0.05$, Fig. 5B) for the first and second cycles following apnea, respectively. T_I was 0.38 ± 0.02 s before AVP and 0.40 ± 0.02 s after high doses of AVP treatment.

Altenuation of AVP-induced Inhibition of PNA by Hypercapnia

Under normocapnia, low and high doses of AVP lowered PNA of the first cycle to 78.20% and 64.16% of the control (Fig. 6A, $P < 0.01$), respectively. The mean PNA was significantly increased with hypercapnia when compared with the control at normocapnia (Fig. 6A, $P < 0.01$) but still reduced by the AVP treatment. However, significant reduction in PNA was only observed with high doses of AVP (Fig. 6A, $P < 0.05$), rather than low doses of AVP (Fig. 6A, $P > 0.05$). This result suggested that the threshold for the AVP-mediated inhibition of PNA was displaced upward at high chemical drive.

Hypercapnia also reduced T_{EE} induced by AVP. For convenience, T_{EE} was transformed into % of the control, i.e., the ratio of T_{EE}/T_E. Low and high doses of AVP treatment prolonged this ratio to 3.68 (Fig.

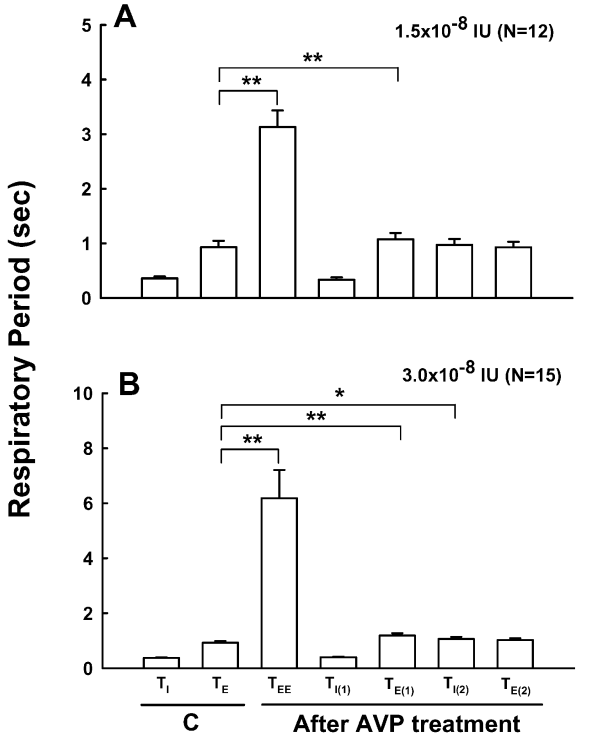

Fig. 5 Respiratory pattern changed with low (A) and high (B) doses of AVP microinjection into the lateral VLM. Only the expiratory period was significantly prolonged with AVP. T_{EE} represented the immediate prolongation of T_E following AVP microinjection. *$P < 0.05$; **$P < 0.01$, compared with the T_E before AVP microinjection. Number in the parenthesis is the first and second neural activity of PNA after resumption from apnea evoked by AVP microinjection. C is the control.

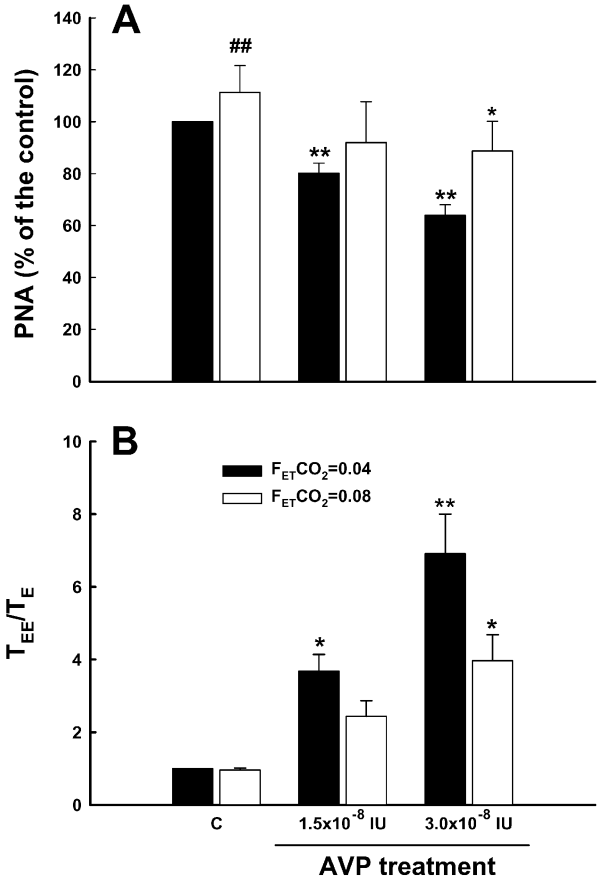

Fig. 6 Dose response of PNA amplitude and apneic period to AVP microinjection was presented. Hypercapnia (blanket bar) increased the amplitude of PNA and thus attenuated the inhibitory effect of AVP on PNA amplitude (A) and the prolongation of apneic period (B). *P < 0.05; **P < 0.01, compared with the control (C) by two-way ANOVA and then with modified t-test. ##P < 0.05, compared with the activity at normocapnia.

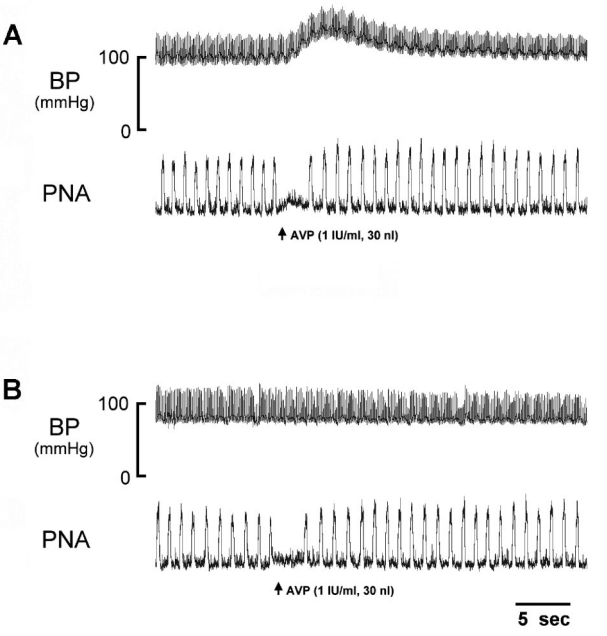

Fig. 7 AVP-activated the lateral VLM produced apnea followed by a decrease of PNA and a concomitant pressor effect (A). Phentolamine abolished the pressor response to AVP activation but not did attenuate the inhibitory modulation on respiration (B).

6B, P < 0.05) and 6.92% (Fig. 6B, P < 0.01), respectively (T_E before AVP administration was 0.97 s). These ratios were shortened to 2.44% (Fig. 6B, P > 0.05) and 3.97% of the control (Fig. 6B, P < 0.05), respectively, in high concentration of CO_2. Hence, attenuation of hypercapnia on the AVP-mediated inhibition of PNA exhibited in both the amplitude and apneic period.

Effect of Increase of BP on the Inhibition of AVP on Respiration

To examine whether the AVP-mediated inhibition of respiration is caused by pressor effect as a consequence of AVP-induced activation in the VLM, PNA response to AVP administration was evaluated before and after venous infusion of phentolamine in three rats. Before pretreatment with phentolamine, AVP injection into the VLM provoked apnea followed by a decrease in PNA and a concomitant pressor effect (Fig. 7A). Venous infusion of phentolamine elicited a decrease in BP. AVP injection following BP recovery from phentolamine infusion could still inhibit PNA without pressor effect (Fig. 7B). A similar reduction in PNA before and after phentolamine was observed in these three animals.

Pressor Response to AVP Microinjection into Lateral VLM

Arginine vasopressin injection into the lateral VLM resulted in hypertension (Fig. 1). The averaged BP was 93.72 and was risen by 7.95 (P > 0.05) and 32.39 mmHg (P < 0.01) with low and high doses of AVP administration, respectively. Heart rate was 397.80 in average and increased by 3.10 (P > 0.05) as well as 3.88 beats/min (P > 0.05) in response to low and high doses of AVP, respectively.

AVP V_{1A}-Receptors and AVP-Induced Cardiopulmonary Responses

Microinjection of high doses of AVP into the lateral VLM produced apnea followed by a decrease in PNA and a concomitant pressor effect (Figs. 8A1). Mean PNA decreased to 68.43% of the control (P < 0.01, Fig. 8B1). High doses of the AVP V_{1A}-receptor antagonist (600 pg) could totally abolish the inhibition of AVP on PNA (Fig. 8A2) and, thus, mean PNA was

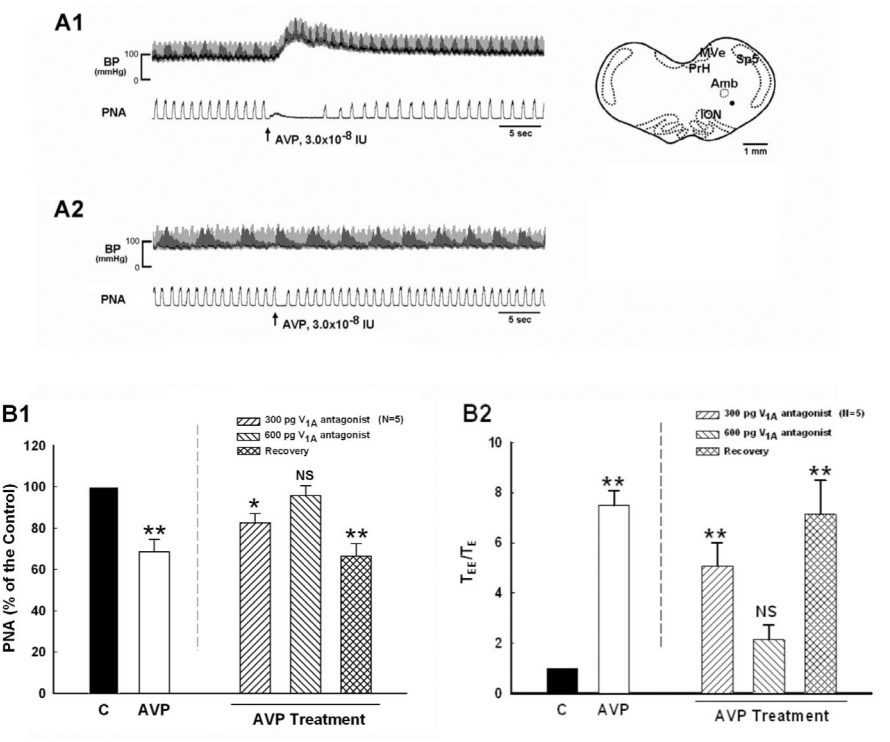

Fig. 8 Cardiopulmonary responses to AVP microinjection are mediated by AVP V_{1A}-receptor antagonist. PNA reduction and pressor effect caused by AVP in one of the animals studied before (A1) and after (A2) pretreatment with AVP receptor antagonist. High doses of antagonist entirely abolished the reduction of PNA (B1) and apneic period (B2) caused by AVP administration. $*P < 0.05$; $**P < 0.01$; ^{NS}nonsignificant, compared with the control (C) by means of ANOVA and then Dunnett's modified *t*-test. See Fig. 1 for abbreviations.

95.71% of the control ($P > 0.05$, Fig. 8B1). Low doses of the antagonist (300 pg) could partially attenuate this inhibition on PNA, showing 82.61% of the control ($P < 0.05$, Fig. 8B1). Microinjection of the AVP V_{1A}-receptor antagonist alone did not induce any effect on cardiopulmonary functions. This antagonizing effect on cardiopulmonary functions by AVP antagonists was reversible. Hence, AVP reduced PNA to 66.47% of the control ($P < 0.01$, Fig. 8B1) 30 minutes later after pretreatment with the antagonist.

The AVP antagonist also reduced T_{EE} caused by AVP injection. The ratio of T_{EE}/T_E was 7.49, in response to AVP treatment before the antagonist ($P < 0.01$, Fig. 8B2), and became 5.08 ($P < 0.01$, Fig. 8B2) and 2.14 ($P > 0.05$, Fig. 8B2) after pretreatment of low and high doses of the antagonist, respectively. Thirty minutes later, AVP microinjection again evoked a significant prolongation of T_{EE}/T_E ($P < 0.01$, Fig. 8B2), indicating that the antagonizing effect of the antagonist is reversible. These data provide evidence that the AVP V_{1A}-receptor antagonist did completely abolish the AVP-mediated effects on both decreases in PNA amplitude and apneic period.

Discussion

The three major bindings in this study are as follows. Firstly, microinjection of AVP into the lateral VLM resulted in an inhibitory effect on respiration and a simultaneous pressor effect. Secondly, this inhibition of PNA with AVP microinjection could be partially attenuated by hypercapnia and was not caused by a baroreflex effect. Thirdly, AVP-induced cardiopulmonary responses are mediated through AVP V_{1A}-receptors, and expressed on neurons located in the lateral VLM.

Microinjection Techniques

Two issues regarding the microinjection techniques should be noted. Firsty, a small volume should be used to reduce bulk diffusion. By delivering a volume of 30 nl of Glu or AVP to the VLM, we could distinguish cardiopulmonary responses from two injection sites with a distance of 0.2 mm apart (Figs. 1 and 2). Thus, pressor area and depressor area of the VLM induced by Glu could be distinguished in a rostra-caudal distribution. This resolution was also displayed in a media-lateral organization, comparing with our recent observation (5). Hence, the VLM probably consists of two areas: one producing pressor effect and the other inducing no pressor response with AVP administration. These two areas distribute in a media-lateral organization. Hence, an injection

volume of 30 nl in our present and recent studies (5) is suitable for investigating the effect of AVP on cardiopulmonary functions. This injection volume is recommended by Lipski *et al*. (17). Secondly, the problem of depolarization blockade caused by Glu excitation on neurons. Whether or not AVP could produce depolarization blockade on neurons as that produced by Glu is unknown. It seems impossible to avoid this depolarization inhibition, if any. Yet, this could be avoided by choosing the lowest concentration of AVP to produce just a threshold change in cardiopulmonary response (5). The reproducible responses of BP and PNA suggest that doses of AVP used in our study did not cause any depolarization blockade or cytotoxicity to neurons. This reproducibility is essential for the evaluation of AVP's effects on respiration at hypercapnia and with the AVP V_{1A}-receptor antagonist. Moreover, activation of neurons by AVP may differ from that by Glu in terms of cellular mechanisms.

Modulation of Cardiopulmonary Functions by AVP-Activated Neurons in Lateral VLM

The area of the lateral VLM involved in AVP modulating cardiopulmonary function is located from the caudal edge of the rVLM to the cVLM, as distinguished by Glu microinjection (2, 3, 5, 13, 19). Histological examinations also revealed that all AVP injection sites were underneath the nucleus ambiguus (NA) and concentrated in a transient area between the compact formation of the NA (NA_C) and loose formation of the NA (NA_L). This area corresponds with the ventral respiratory group (VRG); its respiratory role in the rat has been well documented (4, 7, 18, 29, 38). Furthermore, comparing our study with those reported by other researchers (4, 38), the region we explored with AVP microinjection is located in accordance with the rostral region of the VRG (rVRG). Thus, we have identified an area that is involved in the modulation of cardiopulmonary functions induced by AVP. This area extends from the caudal edge of the rVLM to the cVLM, and corresponds to the rostral regions of the VRG. The present data in conjunction with findings from our recent report (5) indicate that two areas within the VLM, the medial and the lateral, could be excited by AVP. Thus, when activated by AVP, neurons in the medial VLM evoke only an inhibitory action on respiration, while neurons in the lateral VLM elicited a simultaneous inhibitory action on respiration and a pressor effect. Besides, microiontophoretic application of AVP to the VLM has been recently demonstrated to excite and increase the discharge rate of cardiovascular neurons in rats (46).

Little is known about the effect of AVP on respiration, especially its direct action on neurons or areas within the VLM. We have recently reported that AVP could inhibit PNA in the absence of a pressor effect by acting on neurons located in the medial VLM. This is also consistent with the previous findins of Overgaard *et al*. (23), who reported that blockade of AVP receptors could attenuate the increase of ventilation by angiotensin II. Findings in our present study are consistent with those of McCrimmon *et al*. (18), and Chitravanshi and Sapru (4). McCrimmon *et al*. reported that activation of neurons within the rVRG by DL-homocystic acid evoked inhibition of PNA. Chitravanshi and Sapru reported a decrease in the amplitude of PNA when NMDA or Glu was microinjected into the rVRG of rats. In contrast, Kc *et al*. (15) recently reported that microinjection of AVP into the pre-Bötzinger complex (pre-BötC), an area just rostral to the rVRG, increased the electromyographic activity and frequency of the diaphragm. This discrepancy could be partially because the different areas were activated by AVP. The area explored in the present study is located in the rostral region of the VRG, which is just at right caudal to the posterior edge of the pre-BötC. Length of rostro-caudal axis of the pre-BötC is approximately 400 micron (35, 38). However, we did not observe excitatory modulation of AVP on PNA, even with microinjection into sites rostral to the area we studied extensively, as shown in Fig. 2. Nevertheless, microinjections of Glu sometimes induced seizure-like activities and a small upward shift from the baseline of PNA, which is similar to what has been observed by Chitravanshi and Sapru (4). In the study by Kc *et al*., the injection volume was 200 nl, almost seven times larger than what we used in the present study. A large injection volume may result in diffusion and volume expansion. Another possibility for the discrepancy is that experiments by Kc *et al*. were conducted under hypercapnia. High concentration of CO_2 may partially override the inhibitory action of AVP as shown in our data. Moreover, differences in animal strain may also evoke different responses as suggested by Okishia *et al*., (20). Yet whether or not Sprague-Dawley rats and Wistar rats have different responses of cardiopulmonary functions to AVP microinjection is still unknown.

High concentration of $F_{ET}CO_2$ partially antagonized the inhibitory effect of AVP on PNA, not only in amplitude but also in T_{EE}. This could partly be explained by an increase in phrenic discharge, which may lead to a lesser depression of PNA caused by AVP at higher ventilatory drive. It may also be due to an interaction between the inhibitory action of AVP-induced neuron activation and the excitatory effect of chemoreceptors at the level of the VLM. Increases in CO_2 concentration stimulate the carotid

chemoreceptors and central chemoreceptors (19). How signals from these chemoreceptors and the neurons activated by AVP interact is unclear. Based on our data, AVP probably activates neurons at the lateral VLM and in turn inhibit the neurons activated by chemoreceptors. Hence, low doses of AVP at higher ventilatory drive will not be strong enough to initiate these inhibitory mechanisms and would be overridden by the excitation arising from the chemoreceptors.

AVP-Induced Direct Inhibition of Respiration

One may argue that this reduction in PNA could be due to the inhibitory effect of increasing BP caused by AVP treatment. However, PNA was still inhibited by AVP after pretreatment with phentolamine, which effectively prevented the pressor effect caused by AVP. Next, apnea occurred simultaneously with the increase in BP. Specifically, apnea always occurred prior to changes in BP, suggesting that increases in BP probably did not initiate a baroreflex inhibition on respiration. Finally, AVP-activated neurons in the medial VLM did not produce any changes in BP but only a reduction of PNA (5). Hence, we conclude that AVP-producing reduction of PNA must be the consequence of direct activation of AVP V_{1A}-receptors presented on neurons within the VLM rather than an indirect baroreflex effect.

Putative Vasopressinergic Pathway

Our current data suggest that there are intrinsic AVP receptors presented on neurons within the lateral VLM. This finding suggests that AVP activation of neurons, which possess AVP receptors, in the lateral VLM may modulate cardiopulmonary functions. Whether or not AVP excites one group of neurons, which have modulatory actions on cardiopulmonary functions or two distinctive groups of neurons, of which one modulates respiration while the other modulates cardiovascular functions, is unclear.

Based on our data, the AVP receptors found on neurons in the lateral VLM belong to the subtype of V_{1A} receptors. Small doses of AVP V_{1A}-receptors antagonist only partially attenuated the inhibitory effect of AVP on PNA. Large doses of antagonist totally abolished the cardiopulmonary effects induced by AVP. Microinjection of AVP antagonist alone regardless of dosage, did not induce any changes in PNA and BP. Low doses of AVP antagonist may not be enough to saturate all AVP receptors in the injection area. Alternatively, the injected sites of AVP and its antagonist may not be identical, due to the differences in molecular weight of the drugs, a slightly spatial variance of two parallel microelectrodes, and tissue resistance. These problems may be overridden by doubling the injection volume and, hence, a complete blockade of receptors was achieved. Moreover, this antagonizing effect of AVP V_{1A}-receptors antagonist was reproducible and reversible (Fig. 8B). These observations strongly suggest the presence of a vasopressinergic pathway projecting to the lateral VLM.

This putative vasopressinergic pathway could have originated from the PVN, as has been demonstrated from physiological data (22, 33) and histological observations (9, 15). Our data certainly provide a neurophysiological basis for this projection and support the concept of interactions between neurons from the PVN and sympathoexcitatory neurons of the VLM (22, 45, 46).

Physiological Significance

What is the physiological significance of this modulatory role of AVP? Vasopressinergic pathways projecting from the PVN to the VLM is mainly activated by an increase in plasma osmolality. It is unlikely that respiratory activity will be inhibited during the regulation of plasma osmolality. However, AVP has been demonstrated to be involved in the modulation of BP and the redistribution of blood during exercises (14, 21, 37). The release of AVP into the circulation is correlated with the rise in plasma osmolality during submaximal exercises and the release is enhanced with high-intensity exercises. This additional release of AVP is thought to activate the hypothalamic-pituitary-adrenal axis and to maintain ACTH release during prolonged exercises (14). Specifically, this inhibition of respiration induced by AVP was attenuated by hypercapnia. Hence, we may conclude that AVP probably participates in the modulation of respiration during exercises.

To sum up, activation of AVP V_{1A}-receptors on neurons within the lateral VLM by AVP induces an inhibitory effect on respiration and a concomitant pressor effect. This cardiopulmonary modulation may play a role in some physiological conditions such as exercises. More research is needed to elucidate the role of AVP in the modulation of cardiopulmonary functions.

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