Arginine Vasopressin Produces Inhibition upon Respiration without Pressor Effect in the Rat

Ching-Wen Chuang, Meng-Tzu Cheng, Jin-Tun Lin, Hsin-Yun Hsien, Hzi-Yu Hung, and Ji-Chuu Hwang

Department of Biology
National Taiwan Normal University
Taipei, Taiwan, ROC

Abstract

The purpose of the current study was to examine where arginine vasopressin (AVP) inhibits respiration by direct action on the areas of the ventrolateral medulla (VLM) in the rat. The animal was anesthetized by urethane (1.2 g/kg, i.p.), paralyzed with gallamine triethiodide, and artificially ventilated. Catheterization of the femoral artery and vein, and bilateral vagotomy were performed. The rat was then placed upon a stereotaxic instrument in a prone position. The phrenic nerve was separated and cut peripherally. Phrenic nerve activity (PNA) was monitored at normocapnia and hypercapnia in hyperoxia. Microinjection of AVP into various subregions of the VLM was then performed. In response to AVP microinjection, a transient period of apnea and then a significant decrease in PNA amplitude were observed. Arterial blood pressure was unchanged. This inhibition of PNA with AVP treatment was site-specific, attenuated by raising CO_2 concentration, and totally abolished by pretreatment with AVP V_{1A} receptor antagonist. Data of the present study indicate that endogenous resource of AVP may produce an inhibitory effect upon respiration via AVP receptors presented on neurons within the VLM.

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

Introduction

The arginine vasopressin (AVP) is well known as an antidiuretic and pressor hormone, which is synthesized mainly in the paraventricular and supraoptic nuclei in the hypothalamus. The AVP is transported from the hypothalamus, along the axon, to the neurohypophysis, where it is stored. It is released from the neurohypophysis in response to an increase in plasma osmolality or hemorrhage (24, 30). It may play an important role in the maintenance of blood pressure in the situation of dehydration and hemorrhage, and in water balance, in Brattleboro rats (6). Recently, AVP has also been demonstrated to be involved in many other physiological functions, such as memory and learning mechanisms (1), social behavior (21), and reproductive behavior (27). Moreover, there is a preovulatory surge during reproductive cycle (19). Surprisingly, very few reports have paid attention to its modulation on respiration. In this regard, we reported that AVP could increase the airway resistance concomitantly with a reduction of the lung compliance in rats (9). Walker and Jennings (31) also observed that AVP V_1-receptor blockade would substantially enhance the excitation of angiotensin on respiration in conscious dog. However, only a small reduction of respiratory volume with the treatment of AVP V_1-receptor blocker was observed (32, 33, 34). These results suggest that AVP may produce an inhibitory modulation on respiration. Questions still remain as to where is the central location receiving AVP action and what are the mechanisms underlying the inhibitory effect of AVP on respiration. It is conceivable that AVP might produce a direct inhibition upon respiration by acting on some areas in the central nervous system (CNS).

The ventral respiratory group (VRG) in the rat is located underneath the nucleus ambiguus (NA) and

Corresponding author: Ji-Chuu Hwang, Department of Biology, National Taiwan Normal University, Taipei, Taiwan, R.O.C. Tel: 886-2-29332904 ext. 362, Fax: 886-2-29312904, E-mail: jchwang@cc.ntnu.edu.tw
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forms a longitudinal column in the ventrolateral medulla (VLM). It extends from the posterior edge of the facial nucleus to the level of the first spinal cord (4, 14, 23). The VRG plays an important role in the regulation of respiration in the rat. The VLM has been divided into two subareas, which are the rostral ventrolateral medulla (rVLM) and the caudal VLM (cVLM). Excitation to the neurons of the rVLM causes pressor effect, whereas to the cVLM produces depressor effect. In this regard, we have previously reported that chemical activation of the rVLM produced a decrease in phrenic nerve amplitude and an increase in blood pressure (BP) (10). Neurons in the cVLM are also involved in cardiopulmonary modulation (36). Based on these observations mentioned above, we therefore envisaged that the AVP, a pressor hormone and/or peptide, may elicit changes in both BP and respiration when it was directly applied to the area of the VLM. To test this hypothesis, we evaluated the responses of phrenic nerve activity (PNA) and BP to the action of AVP on various areas of the VLM in the rat. The results showed that there was a transient period of apnea followed by a reduction of phrenic nerve amplitude with AVP treatment. Concomitantly, pressor effect was not seen.

Materials and Methods

Animal Preparation

Twenty-seven Wistar rats (300 ± 10 g) of either sex were used in the present study. Atropine (0.5 mg/kg) was given intramuscularly to reduce bronchial secretions. After 30 minutes, the animal was anesthetized with urethane (1.2 g/kg i.p.). Supplementary dosage of urethane (0.12 g/kg, i.v.) was administrated when the animal displayed changes in BP and PNA in response to nociceptive stimuli applying to the paw. Catheterizations of the femoral artery and vein were performed for monitoring blood pressure and administration of drug respectively. BP was monitored with a pressure transducer (Statham P23D, Grass Instrument, Quincy, MA, USA) and recorded in a polygraph chart recorder (Grass, 7D, Grass Instrument, Quincy, MA, USA). Tracheostomy and bilateral vagotomy were performed. The rat was mounted in a prone position in a stereotaxic instrument (Stoeting, Wood, Dale, IL, USA), paralyzed with gallamine triethiodide (Sigma, St. Louis, MO, USA, 5 mg/kg, i.v.), and ventilated artificially. By removing part of the occipital bone and dura mater, the obex was exposed. End-tidal fractional concentrations of CO_2 ($F_{ET}CO_2$) were continuously monitored with a CO_2 analyzer (Electrochemistry, CD3A, Pittsburgh, PA, USA) and were maintained at normocapnia in hyperoxia. Body temperature was maintained at 37-38 °C with the use of a heating pad.

Recording of Phrenic Nerve Activity

The phrenic nerve was dissected dorsally at the level of spinal C_4~C_5 and cut peripherally. The central cut end of the phrenic nerve was placed upon a bipolar electrode. Activity of the phrenic nerve (PNA) was amplified, filtered (0.3~3 kHz) and integrated (R-C circuits) (12). Integrated PNA was displayed on an oscilloscope (Tektronix 5111A, Beavedon, Oregon, USA), recorded on videotape via a PCM (Neuro-Corder 890, New York, NY, USA) and also on the chart recorder (Grass 7D). Some data were stored in hard disc via the PowerLab system (ADInstrument Pty Ltd, NSW, Australia) simultaneously.

Microinjection Technique

A microelectrode with a tip of 2 μm in diameter was infused with AVP by a 32 gauge needle and was connected to a pressure injector (Picosprizer IID, General Valve Corporation, NJ, USA). Microelectrode was fixed on a microelectrode holder, which was mounted on the stereotaxic instrument. The volume of injection was 15 or 30 nl, which was determined by the displacement of fluid meniscus in the microelectrode and was confirmed visually under a binocular microscope (Wild, Heerbrugg, Switzerland) with a ocular meter in one eyepiece. The microelectrode was advanced into various regions of the VLM, which was localized by the coordinates of rat brain atlas (20) and also confirmed by the physiological responses to glutamate injection.

Experimental Protocol

AVP (Sigma) was dissolved in saline to make a solution of 10 IU/ml and stored at -20 °C as stock. This stock solution was then diluted with a saline containing 1% pontamine sky blue during the day of experiment. AVP antagonist (Sigma, [β-mercapto-β, β-cyclopentamethylenepropionyl^1, -O-Me-Try^2, Arg^8]-vasopressin) was dissolved in saline to make a stock with a concentration of 100 μg/ml, which was stored at -20°C. This stock solution was then diluted with saline to a concentration of 10 μg/ml before experiment. Glutamate was dissolved in saline (pH=7.4), which also contained pontamine sky blue to make a concentration of 50 mM.

In a group of five rats, glutamate was injected into various regions of the VLM, the dorsal medulla (DM) and the medullary raphe nucleus (MRN), to identify the location and/or coordinate. In this pilot study, the VLM were distinguished the rostral VLM

from caudal VLM based on the pressor and depressor effects, respectively.

Two protocols were performed in the current study. In the first protocol, two doses of AVP, 1.5×10^{-8} and 3.0×10^{-8} IU, were microinjected into the VLM at normocapnia and hypercapnia in hyperoxia. The injection sequence of AVP was random, low dose followed by high dose in some tries but vise versa in the other animals. Interval of the two AVP injections was at least 30 minutes. The purpose of this protocol was to examine whether AVP exerted an inhibitory action upon respiration. Another purpose was to evaluate whether this inhibition could still be observed under high CO_2 concentration. Responses of BP and integrated PNA were evaluated before and after AVP microinjection. In the second protocol, inhibition of AVP upon respiration was evaluated before and after AVP antagonist administration into the same area. In this study, microinjections were performed through pairs of glass microelectrodes, which were pulled simultaneously with a puller. Tips of microelectrodes were broken and trimmed to a diameter around 20-30 μm under the microscope. This device enables us to make microinjection of two drugs into the same area easily and precisely. With the pairs of microelectrodes, we could inject small amount of AVP via one of the microelectrodes and AVP antagonist through the other.

Data Analysis and Statistical Examinations

PNA on the videotape was played back via PowerLab system and analyzed with software written by Visual C^{++}. Data on the hard disc was directly retrieved and analyzed with software. An average of ten consecutive respiratory cycles before AVP treatment was determined as the control. Neural activities following AVP were chosen as the experimental values and were further transformed into % of the control. All data were expressed as mean ± SEM (standard error of the mean). Average BP before and after AVP treatment were retrieved and analyzed by Data pad module of PowerLab system.

T_I (time for phrenic inspiration), T_E (time between phrenic inspiration) and T_{TOT} (time of total respiratory period or sum of $T_I + T_E$) were also computed before and after AVP treatment.

Data were analyzed by multiple comparisons. Thus, one-way ANOVA was performed and Dunnett's modified *t*-test was then followed (35). BP was evaluated by paired *t*-test (38). Differences between the control and treatment were considered significantly when the *P* value was less than 0.05.

Histological Verification

At the end of the experiment, the rat was sacrificed by an overdose of urethane. The brain stem was then removed and immersed in a 10% formalin solution for at least one week. Fifty μm thick cross section of the brain tissue was made on a frozen microtome. The tissue was then stained with Crystal violet. By using a rat brain atlas (20), microinjection site of AVP was verified. Data outside the specific area were excluded in the present study.

Results

AVP was microinjected into the VLM, DM, and MRN of the brain stem. The reduction of PNA was only discernable in the specific area of the VLM but not in the DM and MRN.

Two Areas of the VLM Could Be Identified by Glutamate

The VLM could be identified into two areas, the rVLM and cVLM, by glutamate excitation to the neurons based on the response of BP. The VLM we explored in this study was a longitudinal area with a coordinate of 1.6-1.8 mm lateral to the midline of the brainstem, 1.2-2.2 mm rostral to the obex, and 1.8-2.4 mm ventral from the surface of the brain stem. As shown in Fig. 1, a conspicuous pressor response was seen from 2.2 mm rostral to the obex as glutamate was injected into the rVLM. A little caudal to this injection site (Fig. 1B and C) glutamate initiated only a minor change in BP. Glutamate-induced excitation to neurons in the area 1.6 mm rostral to the obex evoked an observable depressor effect (Fig. 1D). It appears that a transient area around 1.8 mm rostral to the obex, which was located between the rVLM and cVLM, glutamate produced no change in BP. Amplitudes of PNA were always reduced in response to neuron activation by glutamate no matter in the pressor, depressor, and even transient areas. Changes in respiratory pattern caused by glutamate excitation to neurons in these three areas were somewhat different from each other. A complete inhibition of PNA, showing apnea, was observed in the rVLM (Fig. 1A), whereas incomplete inhibition was seen in the cVLM (Fig. 1D and C). A very different pattern was discernible in the transient area, showing a seizure-like activity. This seizure-like discharge of PNA displayed a combination of several bursts with low amplitude and high frequency, resulting in a upward shift of the baseline of integrated neurogram (Fig. 1B). These phenomena were observed in most of five animals but with variation in either amplitude or frequency. In one of the animals, only one burst with a upward shift of the baseline of neurogram could be seen (similar to that in Fig. 1C).

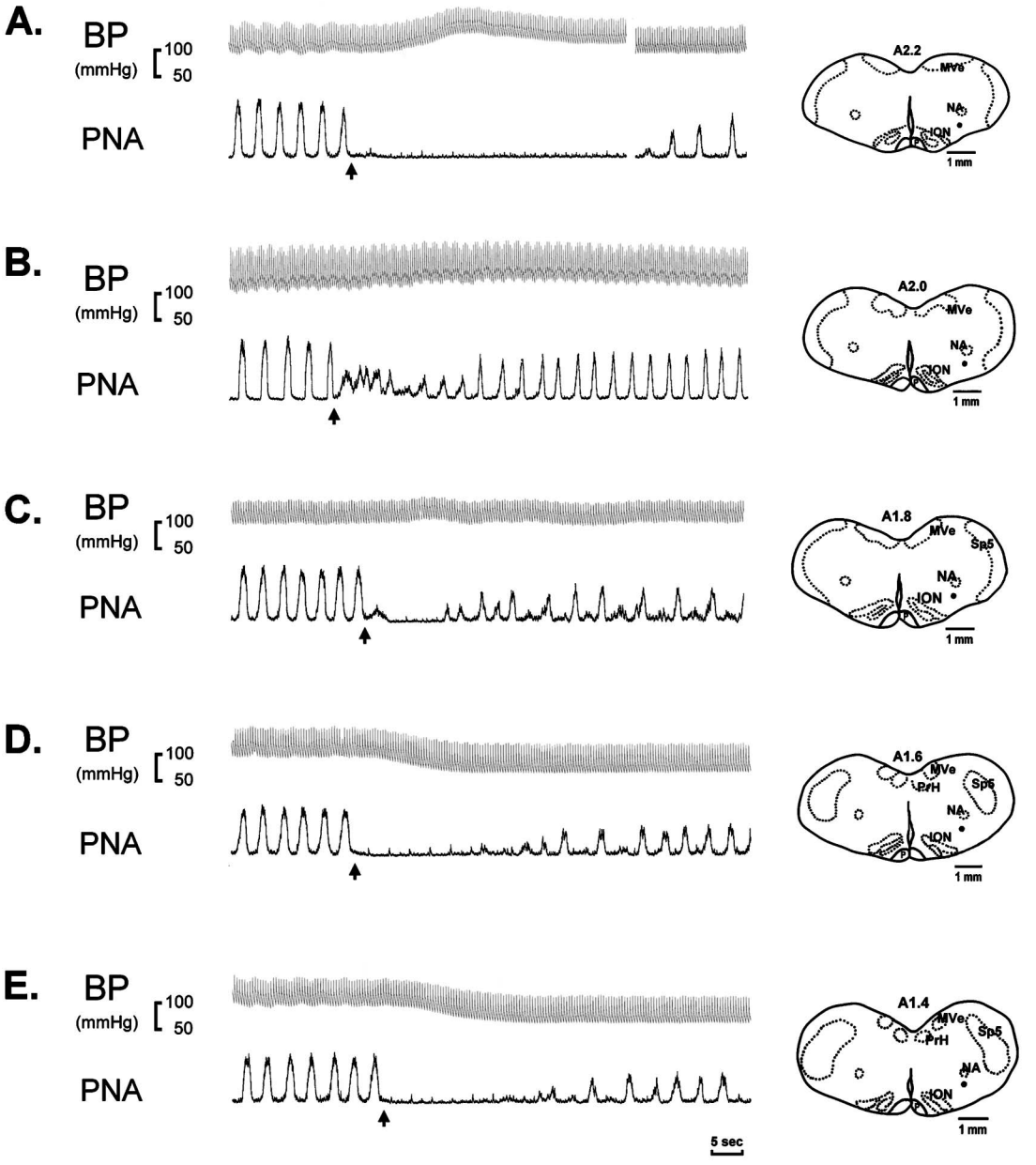

Fig. 1. Example of experimental tracings showing different phrenic and blood pressure (BP) responses to glutamate (upward arrow) administered to different locations of the ventrolateral medulla (VLM). Based on the PB response, the VLM was identified as pressor and depressor areas by glutamate. Concomitant with BP response, phrenic nerve activity (PNA) displayed a transient apnea and then a reduction in amplitude during resumption from apnea when neurons within the VLM were excited. A, rostral to the obex; number following A, distances in mm rostral to the obex; ION ,the inferior olive nucleus; Mve, medial vestibular nucleus; NA, the nucleus ambiguus; P, pyramid; PrH, prepositus hypoglossal nucleus; Sp5 spinal trigeminal tract.

PNA Response to AVP Microinjection into VLM

In response to AVP delivery into the VLM, PNA showed a transient period of apnea and then a reduction in amplitude from resumption of apnea (Fig. 2). The effective area of AVP microinjection located 1.4-1.8 mm rostral to the obex, 1.6-1.8 mm lateral from the midline, and 1.8-2.2 mm ventral from the brain surface. This area corresponded in coordinate with the glutamate-identified transient and cVLM regions. The result different from that of glutamate treatment was that pressor effect was unobservable (Figs. 1 and 2). AVP injection sites outside this small area could not initiate any change in PNA or BP as shown Fig. 2A and B. Sometimes, if injection volume was larger than 30 nl or injection site moved to an area 0.2 mm apart laterally, a pressor response would be observed. The same volume of saline delivery into

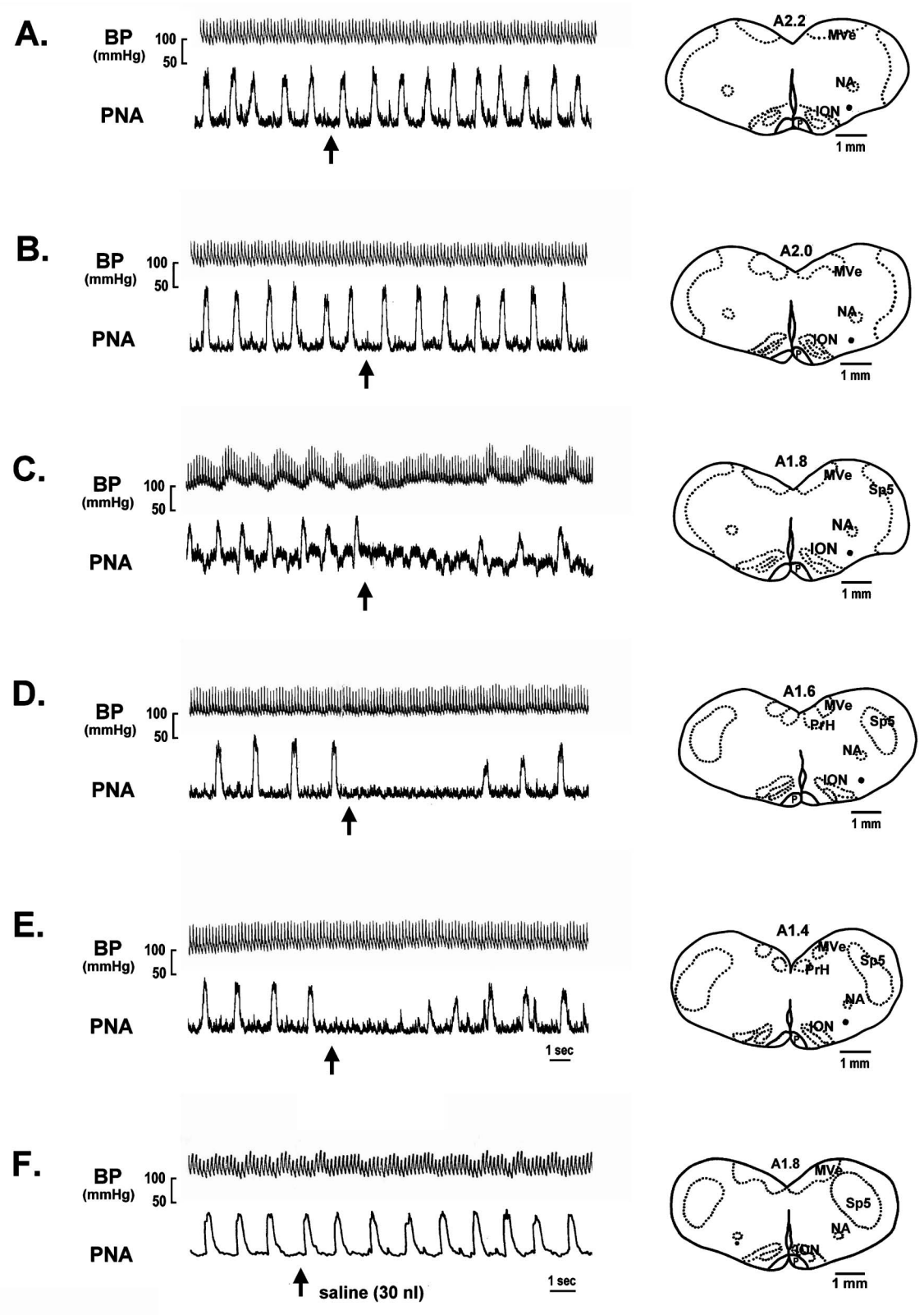

Fig. 2. Experimental tracings showing inhibition of respiration in response to arginine vasopressin (AVP) administered to different lacations of the VLM. Microinjection of AVP into subareas of the VLM produced decreases in PNA. Panels A, B, C, D, E, and F represent different areas of the VLM from 1.8-2.4 mm rostral to the obex, 1.8 mm from midline and 1.8 mm beneath the brain surface. Apnea and decreases in PNA were only seen in area 1.4-1.8 mm rostral to the obex (panel C, D, and E). Microinjection points outside this small area did not produce any change in PNA (panels A and B). Note that changes in BP were unobservable. Saline injection evoked changes in neither PNA nor BP. Uparrow on each panel represents microinjection of AVP with a concentration of 3.0×10^{-8} IU and at a volume of 30 nl. Black spot in the diagrams on the right side of each panel is the injection sites. All the abbreviations are the same as those indicated in Fig. 1.

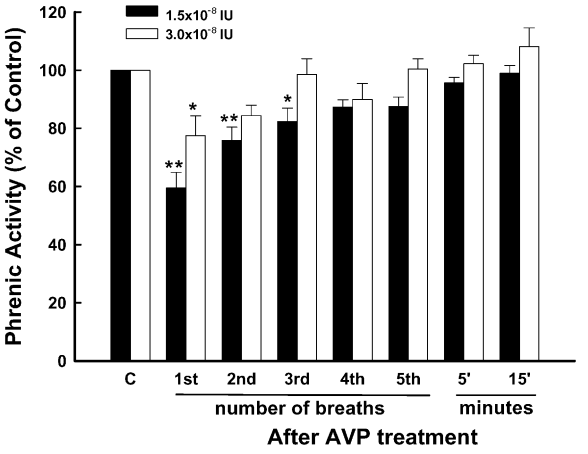

Fig. 3. Time course of PNA responses, showing a gradual recovery from inhibition evoked by AVP treatment. Bars are mean ± SEM. C represents the control, which is average of the consecutive ten neural activities of PNA before AVP treatment. N is the number of observation. *, $P<0.05$; **, $P<0.01$compared with the control (C).

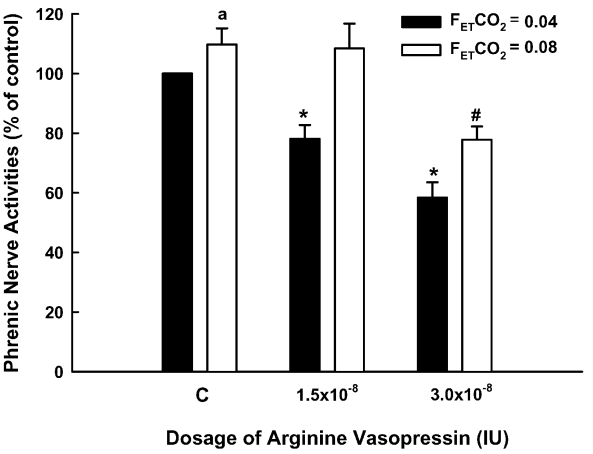

Fig. 4. Dose response of PNA to AVP treatment. Raising the concentration of CO_2 attenuated the inhibition of AVP upon PNA. *a#, $P<0.05$ compared with the control(C) at hyperoxic normocapnia ($F_{ET}CO_2 = 0.04$).

the same effective area of the VLM produced no effect upon either PNA or BP (Fig. 2F).

Seventeen animals were studied in response to AVP treatment and showed a consistent apnea and a decrease in amplitude recovery from apnea. For high dose of AVP treatment (3.0×10^{-8} IU), mean values of the first and second PNA following AVP treatment were 59.49 ± 5.37% and 75.89 ± 4.54 % of the control, respectively (Fig. 3, $P<0.01$). Mean PNA of the third neural breath after AVP was significantly reduced compared to the control (Fig. 3, 82.37 ± 4.59 %, $P<0.05$). Significant reduction of the fourth PNA after AVP was undetectable (Fig. 3). Low dose of AVP (1.5×10^{-8} IU) could also produce significant inhibition on PNA. Yet, compared to the high dose, the extent of reduction in PNA induced by the low dose of AVP was much less and significant reduction of PNA was only seen in the first and second neural breaths (Fig. 3).

Response of PNA to AVP injection into the DM and MRN was examined in five animals. In this experiment, high dose of AVP did not produce any change in PNA or BP.

Inhibition of AVP on PNA Was Attenuated by High Concentration of CO_2

Dose-response for the reaction of PNA to AVP treatment at the effective injection sites was evaluated at normocapnia and hypercapnia in hypermoxia. The immediate response, the first neural activity of PNA after AVP treatment, was selected as experimental value for analyzing effect of dose, and hypercapnia. In this protocol, eight animals were further evaluated at hypercapnia. PNA diminished to 78.17 ± 4.59% of the control (Fig. 4, $P<0.05$) in response to low dose of AVP, whereas it reduced to 58.39 ± 5.12% with high dose (Fig. 4, $P<0.01$). The reduction of PNA to AVP treatment was substantially attenuated by hypercapnia. Hence, AVP increased average PNA to 110.0 ± 5.44% of the control at normocapnia in hyperoxia (Fig. 4, $P<0.05$). At high level of CO_2, a significant reduction of PNA was only discernible for high dose but not for low dose of AVP (Fig. 4). It appears that low dose of AVP-induced PNA inhibition was totally abolished by raising CO_2 concentration.

Changes in Respiratory Pattern in Response to AVP Treatment

T_I and T_E had a tendency to prolong when AVP was microinjected into the VLM. However, significant prolongation of the respiratory period was only seen in T_{EE}, which represented the prolongation of T_E immediately following the delivery of AVP into the VLM. In most of the tests, T_{EE} prolonged immediately after AVP delivery into the VLM. On average, this T_{EE} for low dose of AVP treatment was 4.75 ± 0.61 second, which was statistically significant compared with T_E before AVP treatment (Fig. 5, $P<0.05$, upper panel). T_{EE} for high dose of AVP was 5.71 ± 0.74 second (Fig. 5, $P<0.05$, lower panel). There was no significant difference in T_{EE} between low and high doses of AVP.

AVP Inhibition on PNA Is Mediated via V_{1A} Receptors

Another five animals were evaluated for the

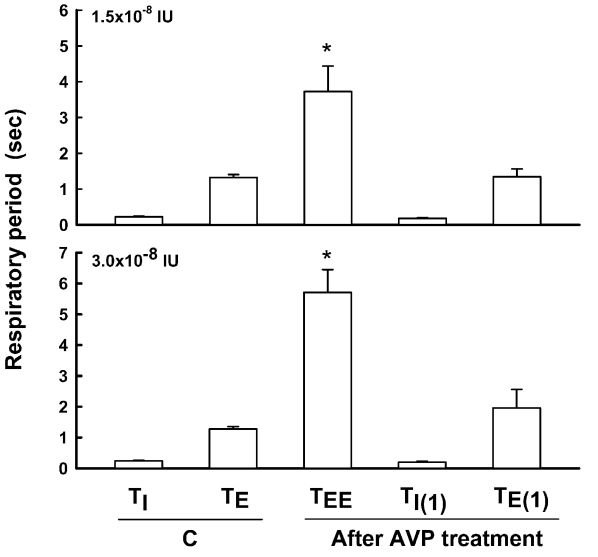

Fig. 5. AVP injection into the VLM produced a change of respiratory period. T_I and T_E represented time for and between phrenic nerve discharged, respectively. T_{EE} represented the prolongation of T_E immediately following AVP treatment. *, P<0.05 compared with T_E of the control (C). The number one in the parenthesis is the first neural activity of PNA after resumption from inhibition evoked by AVP treatment.

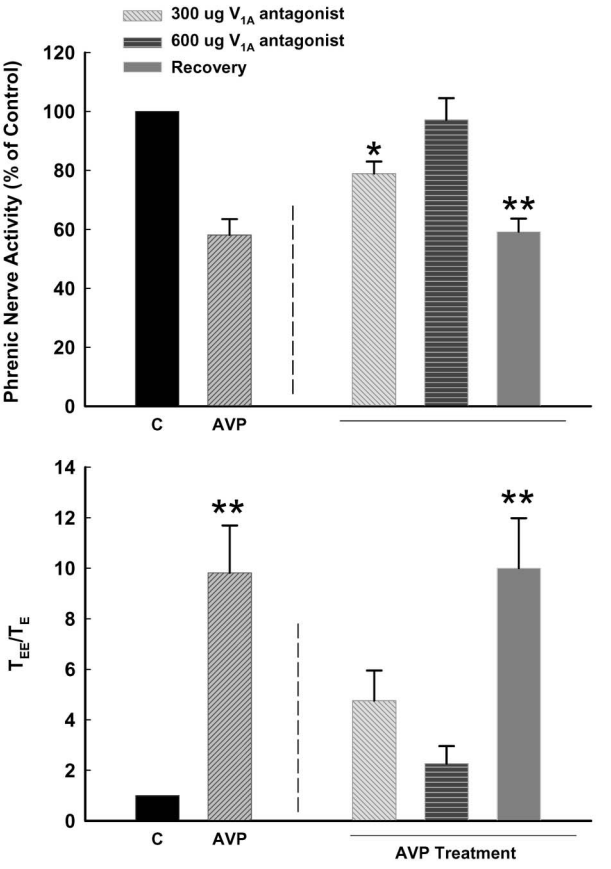

Fig. 6. AVP evoked inhibition upon PNA before (left on dash line) and after (right on dash line) treatment of AVP V_{1A} receptor antagonist. AVP delivery into the VLM produced a significant decrease in PNA (upper panel) and apnea (lower panel). *, P<0.05; **, P<0.01 compared with the control (C). N, observation numbers.

inhibitory effect of AVP on respiration before and after treatment with AVP receptor antagonist. In this protocol, pairs of microelectrodes, one for AVP and the other for AVP receptor antagonist, were used. Only high dose of AVP was used. Hence, AVP delivery to the VLM produced significant decrease in PNA (Fig. 6, 58.10 ± 5.36% of the control, P<0.01). Thirty minutes later, AVP V_{1A} receptor antagonist was microinjected into the same site through the second microelectrode. On examining the effect of antagonist, the same dose of AVP was immediately delivered following the antagonist. Low dose of antagonist (300 μg) attenuated partially the inhibition of AVP upon PNA, whereas higher dose of antagonist (600 μg) totally abolished the inhibitory effect of AVP, showing a 97.19% of the control (Fig. 6. P> 0.05, upper panel) . This antagonized effect of AVP V_{1A} receptor antagonist was reversible and AVP still produced a reduction in PNA (59.24 ± 4.39% of the control, Fig. 6, P<0.01, upper panel) 30 minutes later.

For convenience, apneic response in this experiment was expressed as a ratio, which is T_{EE} after agonist or antagonist treatment divided by T_E before drug treatment. Apneic response prolonged significantly with AVP treatment (Fig. 6, P<0.01, lower panel). This prolongation was antagonized by AVP V_{1A} receptor antagonist. However, 30 minutes after the last antagonist, AVP treatment still evoked a significant prolongation of apneic response (Fig. 6, P <0.01, right side of lower panel), indicating that antagonizing effect is reversible. Accordingly, AVP V_{1A} receptor antagonist abolished the two effects of AVP, decrease in PNA amplitude and apneic period.

Discussion

This study provides the first observation of cardiopulmonary responses induced by the action of AVP upon the VLM. There are three main findings of the present study. First, microinjection of AVP into the VLM could only produce a transient apnea and then a decrease in amplitude of PNA without pressor effect. Second, decrease in PNA with AVP treatment was attenuated by hypercapnia. Third, the inhibition of AVP upon PNA was mediated via V_{1A}-receptor, which is presented on the neurons located within the VLM.

Microinjection

Several aspects must be considered by using microinjection technique. The volume injected should

be small to avoid the diffusion problem. In the present study, the largest volume of AVP delivery to the VLM was 30 nl. At this small volume injection, distance between two effective areas producing different response in BP or PNA by the action of AVP or glutamate was less than 0.2 mm, which could be confirmed from our current results as shown in Figs. 1 and 2. Thus, the rostral VLM of pressor area and caudal VLM of depressor area can be confirmed. Actually, pressor response was observed in an area with a distance of 0.2 mm lateral to this small area we explored (data not shown). Injection volume of AVP larger than 30 nl always evoked pressor effect. This could be caused by a diffusion of AVP to the lateral area with a distance more than 0.2 mm from our effective point. In this regard, Nattie and Li (15) also pointed out that small volume of kainic acid could only produced a reduction of phrenic activity but large volume would initiate changes of both respiration and BP. Due to problem of volume diffusion, data evoked by a volume larger than 30 nl was excluded in the present study. On the other hand, injection volume used in our present study was suitable to investigate the effect of AVP on respiration and this injection volume matched the suggestion by Lipski et al. (11). Another point to be considered is depolarization blockade caused by glutamate injection. We do not know whether AVP could produce depolarization blockade on neurons as that by glutamate. It seems to be unable to avoid the possibility of depolarization inhibition if any. However, we could choice the lowest concentration of AVP to produce just an effective change in PNA. In a pilot study, we tested a variety of concentrations of AVP and found that the lowest concentration to produce significant change in PNA was around 1.5×10^{-8} IU. Moreover, concentration of 3.0×10^{-8} IU of AVP, which was the highest dose used in the present study, could produce reduction in PNA reproducibly. These results indicated that concentrations of AVP used in the present study should not cause any depolarization blockade or cytotoxicity to neurons. This reproducibility was very important for the current study to evaluate the response of PNA to AVP treatment with hypercapnia or antagonist.

Histological examination showed a small spherical spot less than 200 μm in diameter was seen in a single injection point. Trace of dye along the electrode track was not found. Hence, we confirmed that AVP affects only neurons around injection site. Equivalent volume of saline (pH=7.4) injected into the same site did not induce change in PNA. This result indicated changes in PNA must be caused by AVP injection instead of a mechanical effect of volume expansion. Specifically, injection areas outside the VLM, such as the DM and MRN, did not evoke any change in PNA. The DM and MRN were two areas in the brain stem, playing modulation upon respiratory and BP (8, 37). Based on these results, we believed that a specific area located within the VLM must contain receptors or binding sites for AVP. Neurons in this specific area probably play a role in the modulation of respiration when AVP receptors are activated.

AVP Inhibition on Respiration Is Mediated through Specific Area within VLM

The VLM is a rostro-caudal longitudinal area extending from the posterior edge of the facial nucleus to the first spinal cord. Two subdivisions, the rostral ventolateral medulla (rVLM) and the caudal ventrolateral medulla (cVLM), were distinguished based on the response of BP. A body of documents has pointed out that the VLM plays a crucial role in the modulation of cardiopulmonary functions (2, 3, 11, 16). By microinjection of glutamate into to the VLM, we were able to identify these two areas, the result is compatible well with that in the literature (2, 3, 10, 16). Based on the histological examination and a pressor response with glutamate, AVP-induced pulmonary modulation must be located in an area extending from the caudal edge of the rVLM to the cVLM. Thus, the present data showed that AVP injection into the medial depressor area of the VLM produced an observable apnea and then a significant decrease in amplitude of PNA without changes in BP. Data from histology verification also demonstrated that all of AVP injection sites were beneath the nucleus ambiguus (NA) and concentrated at a transient area between the compact formation of the NA (NAC) and loose formation of the NA (NAL). This small area is also corresponded with the ventral respiratory group (VRG), which has recently been well defined in the rat brainstem (4, 5, 23, 14, 29). By comparing the coordinate of the current study with that used by others (4, 29), our small area explored with AVP treatment may be compatible with the rostral region of the rostral VRG (rVRG). Our data also indicate that AVP receptors must be presented in this specific area within the VLM.

AVP is a hormone that is mainly synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus of the hypothalamus and stored at the neurohypophysis. It releases into the circulation in response to the need of physiological conditions such as hemorrhage or hyperosmolality. Recently, this hormone has been documented as neurotransmitters and/or neuromodulators due to its action on a variety of target organs (22). For our understanding, there is no report concerning the inhibition of AVP on PNA by direct action upon neurons or area within the

VLM. We have previously reported that iv infusion of AVP could produce an increase in airway resistance and a decrease in lung compliance (9). Overgaard et al. (18) demonstrated that AVP antagonist could attenuate the increase of ventilation by angiotensin II. Our present results may give rise to a neurophysiological basis to explain the previous data of ours and Overgaard et al. How does AVP produce inhibitory action on respiration by iv infusion remained to be determined. One of the possibilities is that AVP may act indirectly on the central nervous system via the area postrema, an area with incomplete brain-blood-barrier. Moreover, activation of the area postrema did initiate an inhibition upon respiration (28). In the central nervous system, AVP probably excites neurons by binding to AVP receptors. Under this consideration, our present data are compatible well with observations of McCrimmon et al. (14). They found that microinjection of DLH, a powerful excitatory amino acid, into the pre-Bozinger complex and rVRG produced an inhibition upon PNA. Chitravanshi and Sapru (4) reported that there was a decrease in amplitude of PNA with an increase in phrenic frequency when NMDA or glutamate was microinjected into the rVRG. Concomitantly, a decrease in BP was discernible. These results are also comparable with part of our glutamate data but a slight disagreement with our AVP results. This discrepancy could be explained by the activation of different neurons containing various types of receptors located at different sites. Rostral region of the rVRG is just right caudal to the posterior edge of the pre-Bozinger complex. Length of rostro-caudal axis of the pre-Bozinger complex is only around 350~400 μm (26, 29). Thus, we could not exclude the possibility that diffusion of AVP acts upon the neurons of the pre-Bozinger complex. In this regard, parts of our glutamate data showed that PNA has a seizure-like activity and a small upward shift from the baseline, similar to the observation of Chitravanshi and Sapru (4).

Our data demonstrated that high concentration of CO_2 antagonize part of AVP inhibition on PNA. In the current study, bilateral vagotomy was per-formed. Hence, increase in CO_2 concentration must stimulate the carotid chemoreceptor and central chemoreceptors (16). It is not known how signals from both the peripheral and central chemorecptors interact with those from AVP-activated neurons within the VLM. It would be reasonable to consider that AVP probably excites neurons having pathway(s) to inhibit other neurons excited by chemoreceptors. Hence, higher dose of AVP is necessary to recruit or activate more inhibitory mechanisms to override the excitation caused by the chemoreceptors. In term of this consideration, our present data seem to support this assumption.

Putative Pathway of Vasopressinergic Pathway

It is interesting to observe that a vasopressinergic hormone produced an inhibition on PNA but no change in BP when it was injected into the pressor or depressor areas of the VLM. These results suggest that intrinsic AVP receptors presented on neurons of the VLM. They may also indicate that different groups of neurons existed in the brainstem for modulating respiration and cardiovascular functions. The present data show that a small group of neurons within the VLM could be stimulated with AVP and produced apnea without changes in BP. Hence, AVP may be a good neurotransmitter and/or neuromodulator for studying the modulation of pulmonary functions by this small group of neurons within the VLM.

From another point of view, AVP receptors presenting in the area of the VLM or rVRG as shown in our physiological data may suggest that there might be an intrinsic vassopressinergic pathway terminated at this particular area we explored. The putative neural pathway probably originates from the paraventricular nucleus (PVN) in the hypothalamus. This pathway has been recently demonstrated from physiological data (17, 25) and histological observations (7). Our present data certainly give rise to a neurophysiological basis for their imagination and also support a concept of excitation from the PVN to the sympathoexcitatory neurons of the VLM (36, 18) although pressor effect was not observed in this small area. Yet, AVP injection into an area 0.2 mm lateral apart from the area that we explored did produce increase in BP. In this regard, Mack et al. (13) have also reported that a neural pathway projects from the PVN to the rVLM and releases oxytocin to stimulate respiration. Discrepant results could be due to the use of different hormones to act on different neurons. Yet, histochemical data of Mack et al. (13) certainly demonstrated the existence of this neural pathway. Unfortunately, the physiological implications of this pathway remain to be determined.

AVP receptors presenting in neurons within the VLM was further demonstrated by our data, showing that AVP V_{1A} receptor antagonist could abolish the inhibition of AVP upon PNA. Low dose of antagonist with an injection volume of 30 nl could partially abolish the effect of AVP. Higher dose of antagonist, by doubling injection volume to 60 nl in the present study, could totally abolish the inhibition of AVP upon PNA. Injection of antagonist alone, no matter low or high dose, did not produce any change in PNA or BP. Low dose of V_{1A} receptor antagonist may be not strong enough to block all of AVP receptors. It could also be due to a diffusion area different from that initiated by AVP. Increase in dose of antagonist by doubling injection volume may overcome these

two problems and results in total abolition of AVP effect. This may strongly suggest that internal vasopressinergic pathway originating from the PVN to medullary level is really presented and its physiological implication in terms of the modulation of respiration so far is just unclear.

In conclusion, AVP inhibition upon respiration is mediated via V_{1A} receptors located on neurons within the VLM. This inhibition may play a role in modulation upon respiration. It needs more investigations to elucidate how AVP play a modulator role on respiration in terms of physiological implications.

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