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Research Paper

Enhancement of GABA-activated currents by arginine vasopressin in rat dorsal root ganglion neurons

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Abstract: A growing number of studies have shown that arginine vasopressin (AVP) plays an analgesia role in the modulation of nociception. Previous studies have focused on the central mechanisms of AVP analgesia. The aim of the present study was to find out whether peripheral mechanisms are also involved. The effect of AVP on GABA-activated currents (I_{GABA}) and $GABA_A$ receptor function in freshly isolated dorsal root ganglion (DRG) neurons of rats were studied using whole cell patch clamp technique. The result showed that, I_{GABA} were potentiated by pre-treatment with AVP (1×10^{-10}–1×10^{-5} mol/L) in a concentration-dependent manner. Meanwhile, the GABA concentration-response curve was shifted upwards, with an increase of (49.1 ± 4.0)% in the maximal current response but with no significant change in the EC50 values. These results indicate that the enhancing effect is non-competitive. In addition, the effects of AVP on I_{GABA} might be voltage-independent. This potentiation of I_{GABA} induced by AVP was almost completely blocked by the V1a receptor antagonist SR49059 (3×10^{-6} mol/L). Also it could be removed by intracellular dialysis of either GDP-β-S (5×10^{-4} mol/L), a non-hydrolyzable GDP analog, or GF109203X (2×10^{-6} mol/L), a selective protein kinase C (PKC) inhibitor, with the re-patch clamp. These results suggest that AVP up-regulates the function of the $GABA_A$ receptor via G protein-coupled receptors and PKC-dependent signal pathways in rat DRG neurons, and this potentiation may underlie the analgesia induced by AVP.

Key words: arginine vasopressin; GABA-activated current; dorsal root ganglion neurons; patch clamp technique; intracellular dialysis

精氨酸加压素对大鼠背根神经节神经元GABA激活电流的增强作用

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摘 要：越来越多的研究表明，精氨酸加压素(arginine vasopressin, AVP)在痛觉调制中具有镇痛作用。已报道的研究专注于AVP镇痛的中枢作用机制，而本研究旨在研究AVP镇痛的的外周作用机制。应用全细胞膜片钳技术，在急性分离的大鼠背根神经节(DRG)神经元上，观察AVP对GABA激活电流(I_{GABA})的增强作用以及AVP对$GABA_A$受体功能的影响。结果显示，AVP (1×10^{-10}~1×10^{-5} mol/L)预处理后，I_{GABA}增大，GABA剂量效应曲线上移，I_{GABA}的最大值较之对照增加约49.1%；而EC50值几乎不变，表示此种加强为非竞争性的，而且AVP对GABA电流的作用可能是电压非依赖性的。AVP对I_{GABA}的加强作用几乎完全被V1a受体的拮抗剂SR49059 (3×10^{-6} mol/L)阻断。二次钳压技术胞内透析非水解GDP类似物GDP-β-S (5×10^{-4} mol/L)或PKC抑制剂GF109203X (2×10^{-6} mol/L)也可以阻断AVP对I_{GABA}的加强作用。以上结果提示，AVP经由G蛋白耦联受体以及PKC信号通路上调DRG神经元$GABA_A$受体的功能，可能是其诱导镇痛作用的基础。

关键词：精氨酸加压素；GABA激活电流；背根神经节；膜片钳技术；胞内透析
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Arginine vasopressin (AVP) is a nonapeptide of hypothalamic origin that has been postulated to play a regulatory role in nociception through direct activation of central vasopressin receptors and also through receptors that reside in the peripheral tissues [1]. Several studies have also shown that AVP displays anti-nociceptive effects in both humans and animals [2–4]. AVP is synthesized in and secreted by the magnocellular neurosecretory neurons of the hypothalamo-neurohypophysial system [5]. AVP can be used not only as a neurohypophyseal hormone which can regulate peripheral tissue activities but also as a neurotransmitter/neuromodulator in both central and peripheral nervous system (CNS and PNS) [6, 7]. The effect of AVP on antinociception in rat was investigated. Painful stimulus enhances hypothalamic paraventricular nucleus (PVN) synthesis and secretion of AVP, in which there was a negative relationship between pain threshold and AVP concentration [8]. AVP receptors are represented by 3 distinct subtypes classified as V1a, V1b and V2 receptors [9]. The presence of V1a receptor in the rat anterior pituitary was revealed by immunocytochemistry [10, 11]. On one hand, it was shown that V1a receptor in dorsal root ganglia (DRG) might represent a previously unrecognized target for the analgesic action of AVP [12]. On the other hand, both oxytocin (OT) and AVP, as well as the OT and V1a receptor, display a high degree of sequence homology, and both peptides can activate these two receptors [13]. OT-induced analgesia is also mediated by V1a receptor in the mouse [12]. In contrast to OT, AVP modulations of pain are still uncertain and were inferred mostly by mechanism of neuronal V1a receptor [14]. In the spinal cord, OT may inhibit nociceptive neuronal responses indirectly by activating inhibitory GABA interneurons, or directly by inhibiting second-order neurons [15–18]. A recent study has revealed the existence of OT receptor and its modulation on $GABA_A$ receptor function in the primary sensory neurons [19]. The effect of OT on GABA-activated current (I_{GABA}) was mimicked by AVP and mediated by the V1a receptor. Moreover, AVP is expressed in sympathetic ganglia, DRG, trigeminal ganglion (TG) neurons and located in the small cells [20–22]. The functions of neuropeptides in the PNS are diverse, such as sensory transmission, inflammatory response and alarm response [23, 24]. They are also considered to serve as neurotransmitter or modulator in the sensory nervous system [25, 26]. Other reports showed that AVP was related to analgesia in the CNS [27, 28]. It has been shown that AVP release is under a tight regulation by opioids in human and intrathecal injection of AVP fails to produce hyponociception in the tail flick test [20, 29]. AVP-induced analgesic effect on acidosis-evoked pain was completely absent in *V1a*$^{-/-}$ mice, but present in WT littermates [30]. However, it is still unknown whether the effect of AVP on nociceptive processing occurs in peripheral terminals of primary sensory afferents.

γ-aminobutyric acid (GABA) is a major inhibitory transmitter that acts through the $GABA_A$ and $GABA_B$ receptors in the CNS. In the axo-axonal synapses in the spinal cord, GABA and GABA receptors mediate synaptic inhibition by causing a reduction of the release of excitatory transmitter from primary afferent nerve terminal, termed 'presynaptic inhibition' [31, 32]. It has been suggested that a variety of substances modulated the GABA response through phosphorylation and dephosphorylation of the $GABA_A$ receptor-chloride channel complex [33–41]. In the present study, we will investigate the effect of AVP on $GABA_A$ receptor-mediated responses and underlying mechanism in rat DRG neurons.

1 MATERIALS AND METHODS

1.1 *Isolation of the DRG neurons*

The experimental protocol was approved by the Animal Research Ethics Committee of Hubei University of Science and Technology. All procedures conformed to international guidelines on the ethical use of animals, and every effort was made to minimize the number of animals used and their suffering. Six- to eight-week-old Sprague-Dawley male rats were anaesthetized with ethyl ether and then decapitated. The DRGs were taken out and transferred immediately into Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA) at pH 7.4. After the removal of the surrounding connective tissues, the DRGs were minced with fine spring scissors, and the ganglion fragments were placed in a flask containing 5 mL of DMEM, which was composed of 0.5 mg/mL of trypsin (type II-S, Sigma), 1.0 mg/mL of collagenase (type I-A, Sigma) and 0.1 mg/mL of DNase (type IV, Sigma), and incubated at 35 °C in a shaking water bath for 25–30 min. Soybean trypsin inhibitor (1.25 mg/mL of type II-S, Sigma) was then added to stop trypsin digestion. Dissociated neurons were placed into a 35-mm Petri dish and kept for at least another 60 min before electrophysiological recordings. The neurons selected for electrophysiological experiment were 15–35 μm in diameter.

1.2 *Electrophysiological recordings*

Whole-cell patch clamp and voltage-clamp recordings were carried out at room temperature (22–25 °C) by using a MultiClamp-700B amplifier and Digidata-1440A A/D converter (Axon Instruments, CA, USA). Recording pipettes were pulled using a Sutter P-97 puller (Sutter Instruments, CA, USA). The micropipettes were filled with internal solution containing: KCl (140 mmol/L), $MgCl_2$ (2.5 mmol/L), HEPES (10 mmol/L), EGTA (11 mmol/L) and ATP (5 mmol/L). Its pH was adjusted to 7.2 with KOH, and the osmolarity was adjusted to 310 mOsmol/L with sucrose. Cells were bathed in an external solution containing: NaCl (150 mmol/L), KCl (5 mmol/L), $CaCl_2$ (2.5 mmol/L), $MgCl_2$ (2 mmol/L), HEPES (10 mmol/L), *D*-glucose (10 mmol/L). The osmolarity was adjusted to 330 mOsmol/L with sucrose, and the pH was adjusted to 7.4 with NaOH. The resistance of the recording pipette was in the range of 2 to 6 MΩ. A small patch of membrane underneath the tip of the pipette was aspirated to form a gigaseal, and then a negative pressure was applied to rupture it, thus establishing a whole-cell configuration. The adjustments of capacitance and series resistance compensations were done before recording the membrane currents. The membrane voltage was maintained at −60 mV in all voltage-clamp experiments, except when indicated otherwise. Membrane currents were filtered at 2 to 10 kHz, and the data were stored in compatible PC for off-online analysis using the pCLAMP 10 acquisition software (Axon Instruments, CA, USA).

1.3 *Intracellular dialysis by using re-patch technique*

In 're-patch' experiment the first patch-clamp recording was used as the control using a pipette filled with normal internal solution. After recording, the pipette was discarded. On the same neuron, a second patch-clamp recording was performed using another pipette that was filled with normal, GDP-β-S or GF109203X-containing internal solution [38, 42]. After 30 min, the membrane current was recorded again and compared with the results of the control.

1.4 *Drug application*

Drugs including GABA, bicuculline, AVP, SR49059, DMEM, trypsin, collagenase, Dnase and soybean trypsin inhibitor were purchased from Sigma Chemical Co. All drugs were dissolved daily in the external solution just before use and held in a linear array of fused silica tubes (o.d/i.d = 500/200 μm) connected to a series of independent reservoirs. The application pipette tips were positioned ~30 μm away from the recorded neurons. The application of each drug was driven by gravity and controlled by the corresponding valve, and rapid solution exchange could be achieved within about 100 ms by shifting the tubes horizontally with a PC-controlled micromanipulator. Cells were constantly bathed in normal external solution flowing from one tube connected to a larger reservoir between drug applications. In some experiments where GDP-β-S (Sigma) and GF109203X (Research Biochemicals Incorporated) were applied for intracellular dialysis, they were dissolved in the internal solution before use.

1.5 *Data analysis*

All data were analyzed by pCLAMP 10 (Axon Instruments, CA, USA) and Origin 7.5 (Microcal Software, USA). Data were expressed as mean ± SEM. Data were statistically compared using the Student's *t*-test or analysis of variance (ANOVA), followed by Bonferroni's *post hoc* test. Statistical analysis of concentration-response data was performed using the nonlinear curve-fitting program ALLFIT. A P value < 0.05 was considered statistically significant.

2 RESULTS

2.1 Effects of AVP on I_{GABA} in rat DRG neurons

Freshly isolated neurons from rat DRGs were used in the present study. In the majority of the neurons examined (93.8%, 121/129), GABA induced a concentration-dependent (1×10^{-6}–1×10^{-3} mol/L) inward current. The I_{GABA} induced by GABA (1×10^{-4} mol/L) could be blocked reversibly by bicuculline (5×10^{-5} mol/L), a selective antagonist of $GABA_A$ receptor, indicating that this current was mediated by $GABA_A$ receptor (Fig. 1*A*). When GABA was applied regularly for durations of 4 s with 4 min interval, and no obvious 'run-down' of I_{GABA} was seen during the whole-cell recording for at least 90 min. Then, this pattern of GABA application was used in the following experiments.

The 121 DRG neurons sensitive to GABA (1×10^{-4} mol/L) were pretreated with AVP (1×10^{-6} mol/L) for 60 s. The majority (74.4%, 90/121) of I_{GABA} recorded were potentiated obviously by AVP (Fig. 1*B*). The amplitude of I_{GABA} increased by (44.9 ± 6.6)% in 90 neurons ($P < 0.01$, paired *t*-test), and decreased by (12.1 ± 3.1)% in 17 neurons examined. However, AVP had no effect on the I_{GABA} in the other 4 neurons examined (3.3%, 4/121), and the I_{GABA} change in amplitude was

only (2.2 ± 0.9)% ($P > 0.05$, paired *t*-test). In the present study, we established a cut-off value for the effect of AVP, which was a change of I_{GABA} amplitude exceeding 10%. When pooling all data from the 121 neurons examined, pre-application of AVP was found to increase the I_{GABA} by (33.6 ± 3.9)% ($P < 0.05$, paired *t*-test). The potentiating effect of AVP on I_{GABA} disappeared after 8–10 min washout (Fig.1*B*) and was reproducible in the same DRG neurons.

2.2 Blockade of AVP-induced potentiation of I_{GABA} by SR49059

To verify whether the potentiation of I_{GABA} by AVP (1×10^{-6} mol/L) was mediated by the V1a receptor, the effect of the pre-application of AVP with SR49059 (3×10^{-6} mol/L), a selective V1a receptor antagonist, on I_{GABA} was examined. I_{GABA} increased to (144.9 ± 6.6)% with pre-application of AVP under the premise that control currents were normalized to 100% ($P < 0.01$, $n = 7$, paired *t*-test). However, I_{GABA} was (114.1 ± 5.8)% when AVP was co-applied with SR49059 (Fig. 1*D*). As shown in Fig. 1*C* and *D*, the potentiation of I_{GABA} by pretreatment with AVP could be blocked by the administration of SR49059 ($P < 0.01$, $n = 8$, paired *t*-test). Moreover, SR49059 (3×10^{-6} mol/L) itself had no effect on I_{GABA} (data not shown).

2.3 Concentration-dependent potentiation of I_{GABA} by AVP

The potentiation of I_{GABA} was dependent on the concentration of AVP. Fig. 2*A* shows that the amplitudes of I_{GABA} (1×10^{-4} mol/L) increased when AVP was used to pre-treat the DRG at concentrations of 1×10^{-10} mol/L to 1×10^{-5} mol/L. The minimal effective concentration of AVP was 1×10^{-10} mol/L, which produced a (5.4 ± 1.6)% ($n = 8$) potentiation of I_{GABA}. AVP caused the maximum enhancement of I_{GABA} by (46.3 ± 9.4)% ($n = 9$) at concentration of 1×10^{-5} mol/L. The EC50 value of concentration-potentiation curve for AVP was 5.316×10^{-9} mol/L (Fig. 2*B*).

2.4 Effect of pretreatment time of AVP on its potentiation of I_{GABA}

As can be seen from Fig. 3, AVP did not increase I_{GABA} when AVP was applied simultaneously to the DRG

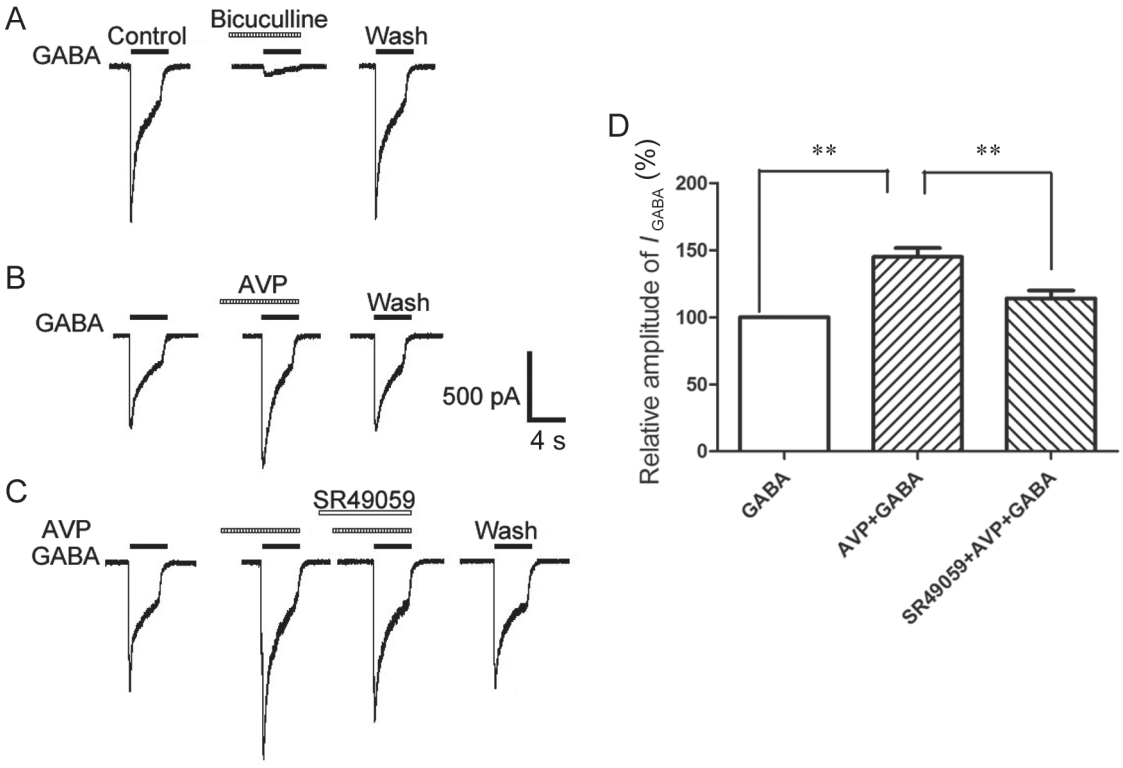

Fig. 1. Modulatory effects of AVP on GABA-activated currents in rat DRG neurons. *A*: The inward current evoked by 1×10^{-4} mol/L GABA could be blocked by the $GABA_A$ receptor antagonist bicuculline in rat DRG neurons evoked at −60 mV. *B*: Sixty seconds of pre-application of AVP (1×10^{-6} mol/L) exerts an enhancing effect on I_{GABA}. The current traces in *C* and the bar graph in *D* show that the potentiation of I_{GABA} by AVP was abolished by SR49059 (3×10^{-6} mol/L), a V1a receptor antagonist. Mean ± SEM, $n = 7$ or 8. $^{**}P < 0.01$.

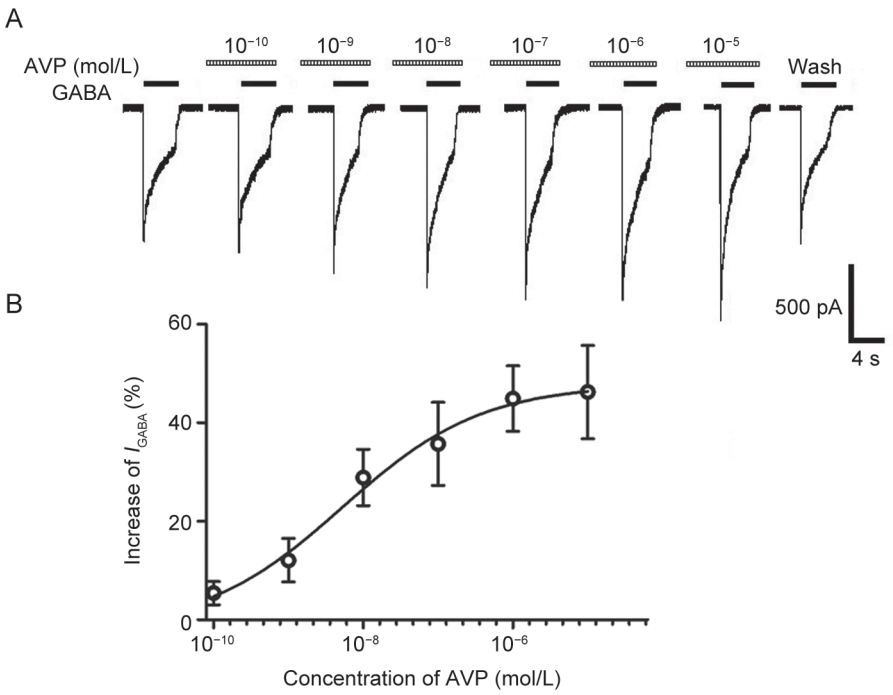

Fig. 2. Concentration-dependent potentiation of GABA-activated currents (I_{GABA}) by AVP. *A*: Sequential current traces illustrating the potentiation of I_{GABA} induced by different concentrations of AVP (1×10^{-10}–1×10^{-5} mol/L) in a DRG neuron. I_{GABA} was elicited by application of GABA (1×10^{-4} mol/L). *B*: Statistical graph showing that I_{GABA} was enhanced step by step with the increase of AVP concentration from 1×10^{-10} mol/L to 1×10^{-5} mol/L. The pretreatment time for AVP was 60 s. Mean ± SEM, n = 6–11.

with GABA (i.e. no pretreatment). However, after pre-application of AVP for at least 15 s, the potentiation of I_{GABA} by AVP emerged. This suggests that the potentiation by AVP of I_{GABA} is a time-dependent process and that an intracellular signal transduction pathway may be involved. To explore how the duration of AVP pretreatment will affect its potentiation on I_{GABA}, AVP at a dose of 1×10^{-6} mol/L was pre-applied to DRG neurons with durations ranging from 15 to 90 s and reached its maximum (53.7 ± 5.2)% (n = 7) with 90 s used. The results depicted in Fig. 3*A* demonstrated that the enhancing effect of AVP on the amplitude of I_{GABA} was increased gradually from 15 s, step by step, until a peak value appeared at 90 s. Thereafter, the I_{GABA} did not increase anymore.

2.5 Concentration-response and current-voltage relationships for GABA with and without pretreatment of AVP

The magnitude of AVP potentiation on I_{GABA} dependeds upon the GABA concentration. Fig. 4*A* shows the concentration-response curves for GABA in the absence and presence of AVP (1×10^{-6} mol/L). It can be seen that (i) the concentration-response curve for GABA with pretreatment of AVP shifted upwards compared with the control; (ii) the EC50 values in both curves were not statistically different ($P > 0.05$, n = 8, Bonferroni's *post hoc* test); (iii) the maximal amplitude of I_{GABA} after pretreatment with AVP increased by (49.1 ± 4.0)% when compared with the control ($P < 0.01$, paired t-test, n = 6); (iv) the threshold values of both curves were basically the same (Fig. 4*A*).

The current-voltage (*I–V*) curves for GABA (1×10^{-4} mol/L) with and without pretreatment with AVP (1×10^{-6} mol/L) were demonstrated in Fig. 4*B*. AVP enhanced I_{GABA} at all holding potentials between −80 and 40 mV as shown by the increase in the slope of the *I–V* curve, and the reversal potentials of two curves were near 0 mV.

2.6 Analysis of intracellular signal transduction pathway in the potentiation of I_{GABA} by AVP

As identified in Fig. 3, the AVP potentiation of I_{GABA} was positively related to the duration of AVP pretreatment, implying that the potentiation was time-consumptive process and that an intracellular signal transduction pathway might be involved. So we further explored the intracellular cascades concerned in the

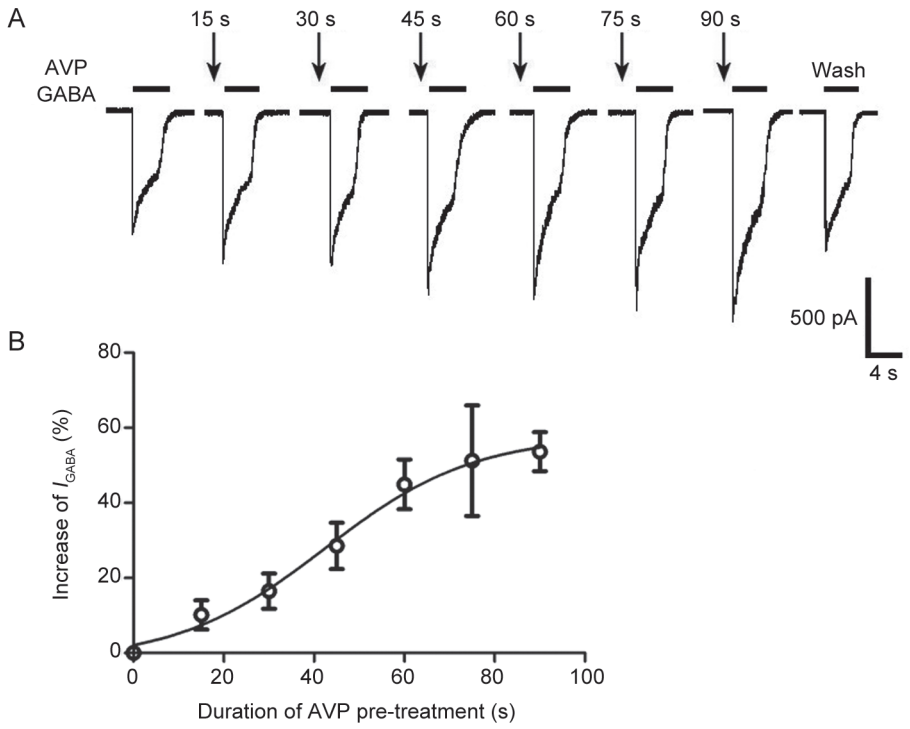

Fig. 3. Effect of the duration of the pre-application of AVP on its potentiation of GABA-activated currents (I_{GABA}). *A*: Current traces demonstrating that the increase of I_{GABA} induced by AVP (1×10^{-6} mol/L) is related to the duration of its pre-application. *B*: Statistical graph showing that the potentiation of I_{GABA} by AVP was enhanced when the duration of the pre-application was increased from 15 to 90 s. Note: Between 75 and 90 s of pre-application, the enhancement of I_{GABA} reached its maximum value. Mean ± SEM, n = 7–10.

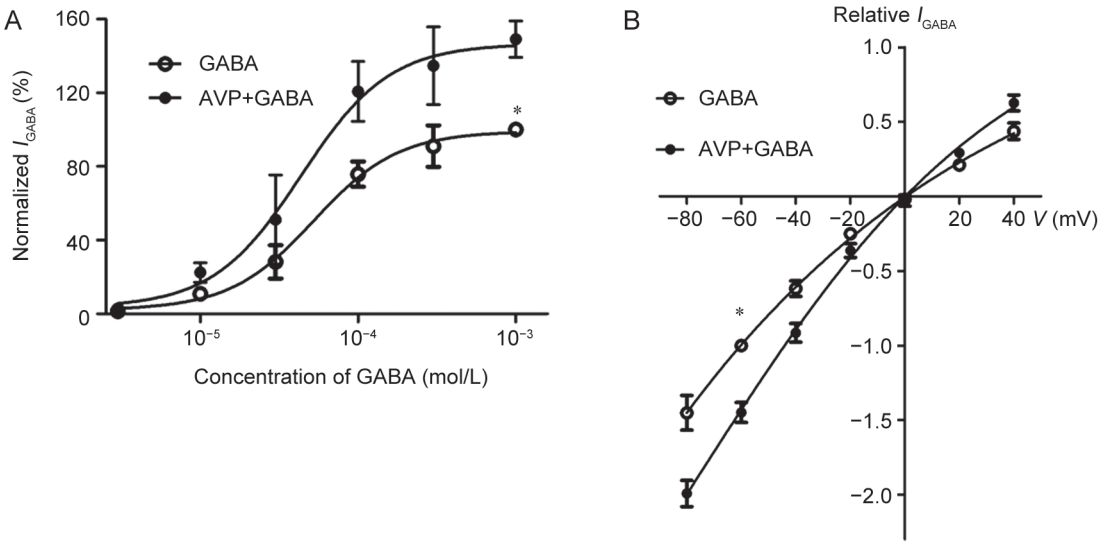

Fig. 4. Concentration-response and current-voltage (*I–V*) relationships for GABA with or without the pre-application of AVP. *A*: The concentration-response curves for GABA with or without AVP (1×10^{-6} mol/L) pre-application. All GABA-induced currents (I_{GABA}) were normalized to the response induced by 1×10^{-3} mol/L GABA applied alone (marked with asterisk). Mean ± SEM, n = 6–9. *B*: The *I–V* curves for I_{GABA} with and without AVP (1×10^{-6} mol/L) pre-application. All current values from the same cell were normalized to the current response induced by GABA (1×10^{-4} mol/L) applied alone at the holding potential of –60 mV (marked with asterisk). Mean ± SEM, n = 6–9. The experiment was carried out using recording pipettes filled with CsCl-containing internal solution.

potentiation of I_{GABA} by AVP using the re-patch technique. V1a receptor belongs to the G protein-coupled receptor family, and the activation of these receptors leads to a cascade of events that activate the PKC system [7, 43]. To further explore whether the potentiation of I_{GABA} by AVP was mediated through the G-protein-PKC signaling pathway, GF109203X (a selective PKC inhibitor, 2×10^{-6} mol/L) and GDP-β-S (a non-hydrolyzable GDP analog, 5×10^{-4} mol/L) were applied internally to DRG neurons through recording patch pipettes. In the control experiments, each neuron was patch-clamped twice with the normal internal solution. When being patched with the pipette filled with normal internal solution for 30 min, the I_{GABA} was (96.4 ± 6.3)% (n = 9). The I_{GABA} was (95.6 ± 9.0)% ($P > 0.05$, *post hoc* Bonferroni's test, n = 12) when the pipette was filled with internal solution containing 2×10^{-6} mol/L GF109203X, and the I_{GABA} was (98.3 ± 7.2)% ($P > 0.05$, *post hoc* Bonferroni's test, n = 7) when the internal solution contained GDP-β-S (5×10^{-4} mol/L) (Fig. 5*B*). In contrast, the potentiation of I_{GABA} induced by AVP was abolished when examined at 30 min after intracellular dialysis with either GF109203X ($P < 0.05$, n = 9, *post hoc* Bonferroni's test) or GDP-β-S ($P < 0.01$, n = 9, *post hoc* Bonferroni's test) (Fig. 5*C*). Taken together, these results suggest that the binding activity of AVP to the V1a receptor may be reduced by PKC inhibitors.

3 DISCUSSION

The present study demonstrated that AVP dose-dependently enhanced I_{GABA} via V1a receptor and PKC dependent signal pathways in freshly isolated rat DRG neurons. AVP shifted the GABA concentration-

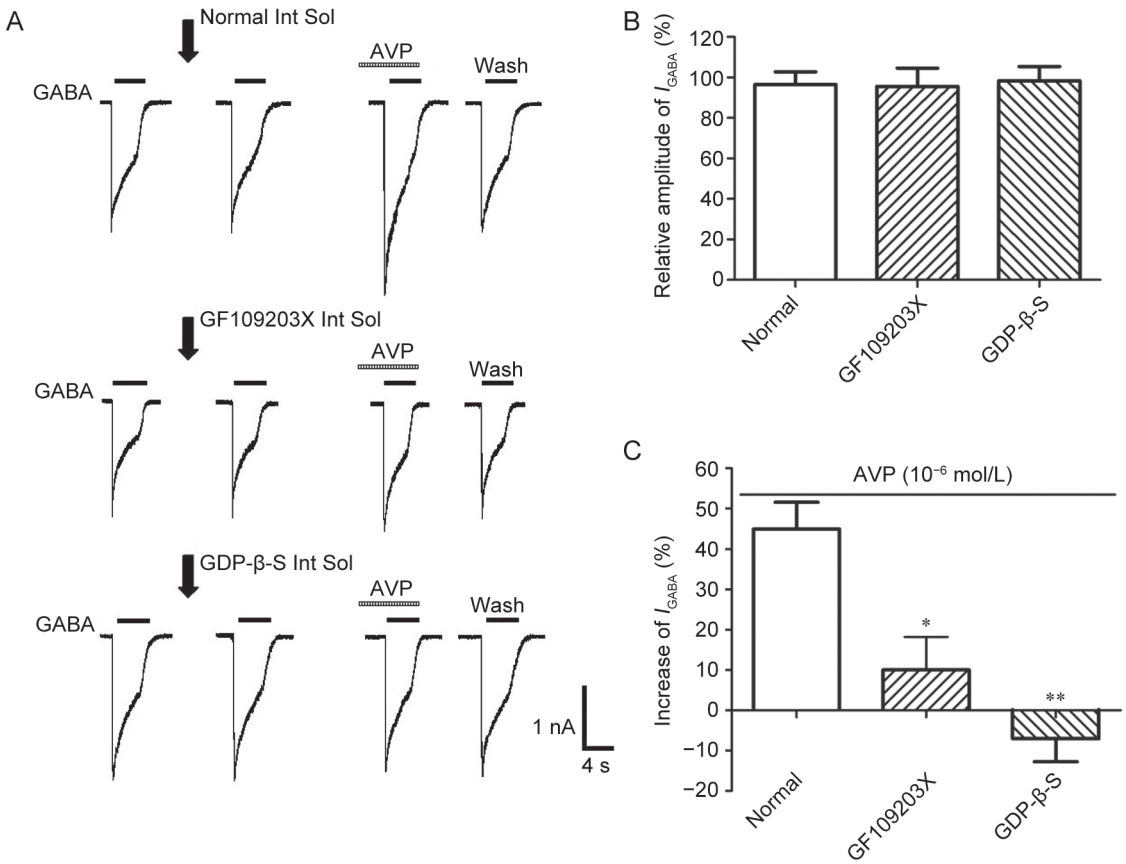

Fig. 5. G-protein coupling and intracellular phosphorylation are shown to be involved in the potentiation of GABA-activated currents (I_{GABA}) by AVP. *A*: Current traces after treatments with normal internal solution (Int Sol), GF109203X (2×10^{-6} mol/L), or GDP-β-S (5×10^{-4} mol/L). GF109203X and GDP-β-S were included in the recording pipette for intracellular dialysis. Arrows represent re-patch clamp with normal internal solution or intracellular dialysis. *B*: Statistical graph showing that GF109203X (n = 12) or GDP-β-S (n = 7) alone had no effect on I_{GABA}, compared with normal internal solution (n = 9). *C*: Intracellular dialysis of GF109203X or GDP-β-S abolished the enhancing effect of AVP (1×10^{-6} mol/L) on I_{GABA}. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* Normal. Mean ± SEM, n = 9.

response curve upwards and increased the maximum response without changing the threshold value and EC50 significantly. These results showed that the intrinsic efficacy of the $GABA_A$ receptor increased after it was pretreated with AVP. However, its affinity did not change. This also indicated that AVP did not act at the recognition site for GABA on the $GABA_A$ receptor and enhanced I_{GABA} in a non-competitive manner. Moreover, the AVP-induced potentiation of I_{GABA} might not be voltage-dependent. There results suggest that AVP activated the signal pathway of V1a receptor-G-protein-PKC in DRG neurons. The minimum effective concentration for AVP to cause GABA-activated currents responses was 1×10^{-10} mol/L in DRG neurons. This concentration is close to that reported for AVP-evoked intracellular Ca^{2+} concentration responses in hippocampal and cortical astrocytes [44]. Moriya *et al.* also reported that AVP (1×10^{-10}–1×10^{-6} mol/L) induced an increase in intracellular Ca^{2+} in the non-neuronal cells isolated from the rat DRG and cultured *in vitro*, and the minimum effective concentration of AVP was 1×10^{-10} mol/L [5]. Thus we demonstrated that I_{GABA} of DRG neurons responses to AVP in a concentration-dependent manner in the physiological range. These results also suggest that AVP modulates the activity of DRG glial cells via activation of V1a receptor.

The $GABA_A$ receptor belongs to a superfamily of ligand-gated ion channel receptors whose intracellular loop between transmembrane domains III and IV is the target of many protein kinases, and the phosphorylation of these receptors can result in receptor function changes [45]. $GABA_A$ receptor is down-regulated by direct phosphorylation via PKC [46]. It has been established that the suppression of I_{GABA} by substance P (SP) is caused by phosphorylation of $GABA_A$ receptor through activating PKC [41, 46]. It was also regarded that potentiation of $I_{5\text{-HT}}$ by PKC is mediated by the promotion of membrane trafficking of 5-HT_3 receptor via F-actin [47]. Recent studies have demonstrated that AVP increases GABA to release V1a receptor and the effects of vasopressin on GABAergic transmission in the hippocampus via G-protein, intracellular Ca^{2+} and PKC [48]. In the present study, we found that the potentiation of I_{GABA} by AVP was blocked by SR49059, a V1a receptor antagonist [49]. Moreover, the AVP applied prior to GABA application induced potentiation of I_{GABA}, and this effect was positively related to the duration of AVP pretreatment, implying that this potentiation was a time-consuming process. Thus, the potentiation of I_{GABA} by AVP possibly involved intracellular signal transduction. GDP-β-S, a non-hydrolyzable substrate for G-protein, prevented the enhancing effect of AVP on the I_{GABA}, suggesting that a G-protein pathway mediated the potentiation of the native I_{GABA} in DRG neurons. The potentiation of I_{GABA} was clearly blocked by intracellular dialysis of GF109203X, a selective PKC inhibitor [50]. In addition, it has been shown that GABA-receptor-mediated current increased when cAMP-dependent protein kinases were inhibited by the activation of the CB1 receptor [38]. Thus, the $GABA_A$ receptor-mediated current increased when these kinases were inhibited by the activation of the V1a receptor. These results suggest that AVP activated the signal pathway of V1a receptor-G-protein-PKC. These results help to extend our understanding of the relevant receptor physiology. It should be pointed out that rich sources of synthetic ligands and knowledge of ligand-receptor interactions allowed the AVP/OT receptor system to act as a prototypical G-protein-coupled receptors family member.

Schorscher-Petcu *et al.* concluded that V1a receptor mRNA was abundantly expressed in mouse DRG neurons, and V1a receptor positive neurons were predominantly of small and medium diameter [12]. Similar to OT, systemic injections of AVP resulted in analgesia [51]. OT and AVP can not cross the blood-brain barrier [52]. So far, it is not known whether the hormonal role of AVP on nociceptive processing occurs in peripheral terminals of primary sensory afferents. Several reports have provided evidence for the presence of $GABA_A$ receptors in the primary sensory neurons of adult rats. For example, the expression of $GABA_A$ receptor in the soma and central processes of nociceptive DRG and TG neurons was reported in the adult rat [53, 54]. A study has also shown that OT inhibits the activity of acid-sensing ion channels through the V1a receptors in primary sensory neurons in the rat DRG neurons [30]. As we know, $GABA_A$ receptor belongs to the superfamily of ligand-gated ion channel receptors, while $GABA_B$ receptor is classified as a G-protein-coupled receptor [55]. Our results revealed that the function of the $GABA_A$ receptors was potentiated by the activation of V1a receptors in primary sensory neurons, indicating that the analgesic effect of AVP originated in the peripheral terminals of primary sensory neurons via the activation of $GABA_A$ receptors expressed in the peripheral terminals of the DRG neurons. However, GABA acts on both $GABA_A$ and $GABA_B$ receptors, so we cannot exclude the possibility that $GABA_B$ receptors can be

potentiated by the activation of V1a receptors in primary sensory neurons. To confirm this point, we need more research.

GABA is an established inhibitory neurotransmitter that acts through the $GABA_A$ receptors. It opens the Cl^- channel and is involved in primary afferent depolarization, an effect known as 'pre-synaptic inhibition' [31]. This action of GABA results in a decrease in the amount of neurotransmitter, including SP and glutamate released from primary afferent terminals [38]. Under normal conditions, GABA exerts tonic modulation of nociceptive neurotransmission between primary afferents and second-order, spino-thalamic tract neurons [56]. In the present study, we used the cell body of DRG neurons as a simple and accessible model to examine the characteristics of the membrane of peripheral terminals. The presence of AVP and a neuropeptidergic system in DRG has been known for some time [57, 58], but there are no detailed studies of the possible effects and signaling pathways evoked by involvement AVP. If AVP enhances the GABA response at the peripheral terminals of primary afferent neurons by activating the V1a receptor, as it does in the soma membrane, potentiation of the 'pre-synaptic inhibition' would directly result in the inhibition of nociception in the spinal cord. It is known that the analgesic actions of exogenously administered neurohypophyseal hormones target at different pain modalities [1, 59–62], but very little is known about the role of endogenous AVP in pain processing. Therefore, AVP was directly associated with the modulation of primary sensory information (including pain) at the peripheral terminals of primary afferent neurons, which provides a reasonable explanation of AVP-induced antinociception in the spinal dorsal horn.

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