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Original Articles

Modulation of *Corydalis tuber* on Glycine-induced Ion Current in Acutely Dissociated Rat Periaqueductal Gray Neuron

Byung-Shik Cheong, Sang-Soo Nam, Do-Young Choi

Department of Acupuncture and Moxibustion, College of Oriental Medicine, Kyung Hee University

This study was designed to investigate the modulation of the *Corydalis tuber* on glycine-activated ion current in rat periaqueductal gray (PAG) neurons.

Aqueous extract from *Corydalis tuber* has been widely used for pain control such as dysmenorrhea, irregular menstruation or amenorrhea with abdominal cramping, neuralgia, headache and gastrointestinal spasm. The PAG region of the brain is known to be involved heavily with nociception.

Modulation of the *Corydalis tuber* on glycine-induced ion current in rat periaqueductal gray (PAG) neurons was studied by a nystatin-perforated patch-clamp technique.

High concentrations of *Corydalis tuber* elicited ion current, which was suppressed by strychnine application. Low concentrations of *Corydalis tuber* reduced glycine-induced ion currents in the PAG neurons.

Inhibitory action of *Corydalis tuber* on glycine-activated ion current was reduced by treatment with naltrexone, a non-selective opioid antagonist. Application of N-methylmalemide (NEM), a sulfhydryl alkylating agent, also reduced the inhibitory action of *Corydalis tuber* on glycine-activated ion current in the PAG neurons.

These results suggest that the inhibitory effect of *Corydalis tuber* on glycine-activated ion current in the PAG neurons is one of the analgesic mechanisms of the *Corydalis tuber*, which may activate descending pain control system in PAG neurons.
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Key Words: *Corydalis tuber*, glycine, periaqueductal gray neurons, opioid, G- protein

Introduction

Corydalis tuber, the root of *Corydalis yanhusuo* W. T. Wang, is considered in Oriental medicine chiefly as an analgesic to help invigorate the blood and relieve almost any painful condition[1,2,4]. The analgesic and sedative properties of the *Corydalis tuber* are essentially ascribed to several alkaloids: DL-tetrahydropalmatine (THP), D-corydaline, and corydalis H, I, J, K and L. The alkaloids exhibited analgesic, anti-arrhythmic, anti-thrombic, anti-inflammatory, anti-cataract, anti-hypertensive and antiallergic activities[5]. Among them, THP is a very effective monoamine depletor in the brain and also possesses analgesic, sedative, hypnotic, antiepileptogenic and anticonvulsant agents[6].

The transmission of nociceptive information may be altered by various neuronal circuits within the central nervous system (CNS). One of them is the descending pain control system, which consists of three major

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Correspondence to:Do-Young Choi, Department of Acupuncture and Moxibustion Kyung Hee Oriental Medical Center Hoegi-dong, Dongdaemun-gu, Seoul, Korea; Tel: 82-2-958-9206, Fax: 82-2-957-4245, E-mail: choi4532@unitel.co.kr

components: the periaqueductal gray (PAG) of the midbrain, the rostroventral medulla (RM) including the nucleus raphe magnus, and the spinal dorsal horn. Of these pain control systems, modulation of pain in the PAG matter is the most extensively studied[7,8]. PAG is known as a major target of analgesic action of the opioid in the CNS[9]. Stimulation within the midbrain PAG produces an opioid receptor-mediated analgesia[10].

Opioid peptides and opiates produce analgesia by activating the descending pain modulatory pathways, especially at the level of the PAG[7,11,12]. Opioid peptides and opiates regulate the nociceptive transmission in part by inhibiting the release of transmitters[11,13]. The effects of opiates and opioid peptides also have been reported to activate potassium channels[14,15] or to inhibit calcium channels[16,17]. It is proposed that endogenous opioid peptides can activate PAG output neurons by inhibiting inhibitory interneurons[8].

The amino acid glycine is a major inhibitory neurotransmitter in the brainstem and spinal cord. The inhibitory action of glycine is mediated by a strychnine-sensitive glycine receptor and a glycine-gated chloride ion channel. Inhibitory glycine synapses in the brain stem and spinal cord are closely implicated in the transmission of nociception, in which glycine inhibits neurotransmission and relieves pain. In addition, glycine-mediated inhibitory effect induces muscle relaxation, whereas inhibition of glycine receptors by strychnine induces convulsion[18,19].

Although *Corydalis tuber* is known to be involved in pain, the effect of *Corydalis tuber* on neuronal activity at the level of PAG has not been yet reported. In this study, the modulation of *Corydalis tuber* on glycine-induced ion current in the acutely dissociated PAG neurons was investigated using nystatin-perforated patch-clamp technique under voltage-clamp condition.

Materials and Methods

1. Preparation of PAG Neurons

The PAG neurons were freshly dissociated using a technique described previously elsewhere[12,16]. In brief, 10- to 15-day-old Sprague-Dawley rats of both sexes were decapitated under Zoletil 50® anesthesia (50 mg/kg; im). The brain was removed and the transverse slices (400 ㎛ thickness) were made with a microslicer (DTK-1000, DSK, Tokyo, Japan). Slices were preincubated in the incubation solution that had been well saturated with 95% O_2 and 5% CO_2 at room temperature for 30 min. Then, the slices were treated with pronase (protease XIV, 1 mg/6 ml of the oxygenated incubation solution) for 40 - 80 min at 32 ℃ and subsequently with thermolysin (protease X, 1 mg/6 ml) for 10 - 20 min at 32 ℃. After enzyme treatment, the slices were kept in the enzyme free incubation solution for 1 h. PAG region was identified in a 60 mm culture dish coated with silicone under a binocular microscope (SZ-ST, Olympus, Tokyo, Japan), and was micropunched out from the slices with an electrolytically polished injection needle. The micropunched PAG regions were mechanically dissociated in a different dish with fire-polished fine glass Pasteur pipettes in 35 mm plastic culture dishes (3801, Falcon, Franklin Lakes, NJ) filled with standard solution. The dissociation procedure was done under an inverted phase-contrast microscope (CK-2, Olympus, Tokyo, Japan). The dissociated neurons usually adhered to the bottom of the dish within 20 min. These cells were remained viable for electrophysiological studies up to 6 h after dissociation.

2. Solutions

The ionic composition of the incubation solutions was (in mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, glucose 10, and $NaHCO_3$ 24. The pH

was adjusted to 7.4 by continuous bubbling with 95% O_2 and 5% CO_2. The composition of the standard external solution was (in mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and *N*-2-hydroxyethylpiperazine-*N'* -2-ethanesulphonic acid (HEPES) 10. The pH was adjusted to the 7.4 with tris-hydroxymethylaminomethane (Tris-base). The composition of the internal pipette solution for nystatin perforated recording contained (in mM) KCl 150 and HEPES 10. The pH was adjusted to 7.2 by adding Tris-base. A stock solution containing 10 mg/ml nystatin in methanol was prepared and added in a final concentration of 200 ㎍/ml to the patch pipette solution.

3. Drugs

Corydalis tuber used in this experiment was obtained from the Kyungdong market (Seoul, Korea). After washing, *Corydalis tuber* was immersed in cold water for 12 h. In order to obtain aqueous extracts of *Corydalis tuber*, it was subsequently heat-extracted, pressure-filtered, and concentrated with a rotary evaporator. The resulting 32 g of powder (yield of 16 %) was obtained from 300 g of *Corydalis tuber* through lyophilization by drying machine for 24 h.Zoletil 50® were obtained from Vibac Laboratories (Carros, France) and most drugs used in this study including nystatin, strychnine, glycine, N-methylmalemide (NEM), and naltrexone were purchased from Sigma Chemical Co. (St. Louis, MO). Drugs were added to the standard solution at the final concentrations provided in the text and were applied using a rapid application system termed the "Y-tube method" as described elsewhere[14,20]. By this technique, the standard solution surrounding a neuron could be exchanged within 10 - 20 ms.

4. Electrical Measurements

Electrical recordings were performed in the nystatin-perforated patch recording mode under voltage-clamp conditions. Patch pipettes were prepared from glass capillaries with an outer diameter of 1.5 mm on a two-stage puller (PB-7, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with the internal pipette solution and the reference electrode was 6 - 8 MΩ. After stable perforated patch formation, the series resistance ranged from 16 to 25 MΩ. Electrical stimulation, current recordings, and filtration of currents (at 2.9 kHz) were obtained with an EPC-7 patch-clamp amplifier (List-Electronic, Darmstadt/Eberstat, Germany). The current and voltage were monitored on a pen recorder (Recti-Horiz-8K, NEC San-ei, Tokyo, Japan). All experiments were performed at room temperature (22 - 24 ℃).

5. Data Analysis

Results are presented as mean ± standard error mean (S.E.M.); Student' s *t*-test was used for statistical analysis and *P*-values less than 0.05 were considered significant.

Results

1. Ion Currents Activated by *Corydalis tuber*

In the nystatin-perforated patch-clamp mode, experiments were carried out at a holding potential (VH) of -50 mV. *Corydalis tuber* was applied every 2 min and ion current activated by 1 mg/ml *Corydalis tuber* used as a control. Inward currents were recorded by *Corydalis tuber* at various concentrations. The concentration of 0.05 mg/ml of *Corydalis tuber* did not elicit an ion current, while application of 0.1 mg/ml, 0.5 mg/ml, 3 mg/ml, and 5 mg/ml of *Corydalis tuber* elicited ion currents of 1.82 ± 1.84 % (n=6, $p<0.05$), 57.91 ± 2.82 % (n=6, $p<0.05$), 158.19 ± 2.93 % (n=6, $p<0.05$) and 243.52 ± 4.44 % (n=6, $p<0.05$) of the control value, respectively. *Corydalis tuber* thus elicited

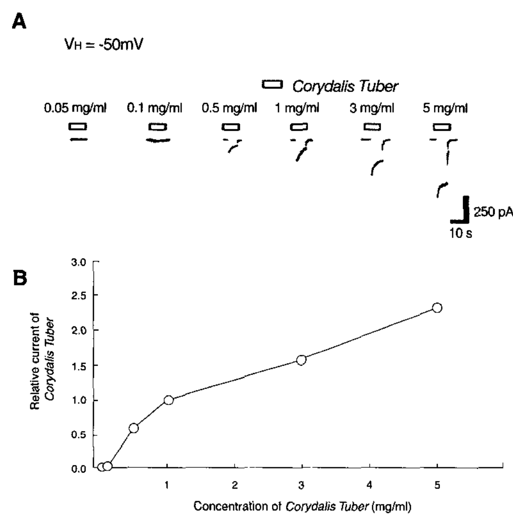

Fig. 1. Ion currents activated by *Corydalis tuber*
Nystatin-perforated patch-clamp under voltage clamp condition (VH = -50 mV) was performed on acutely dissociated periaqueductal gray (PAG) neuron. *Corydalis tuber* was applied every 2 min and resultant ion currents were measured. *Corydalis tuber* activated ion currents in a concentration-dependent manner in PAG neurons.

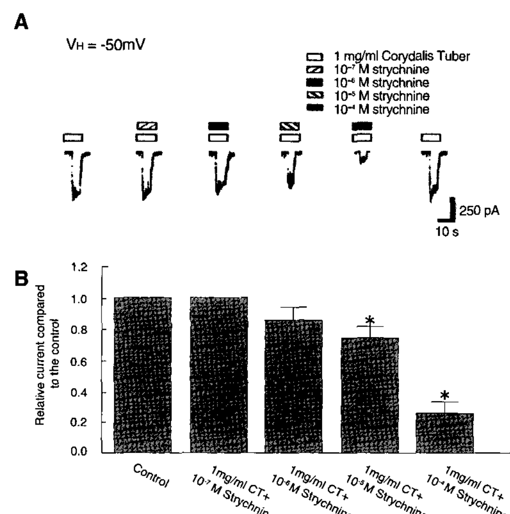

Fig. 2. Effect of strychnine on *Corydalis tuber*-activated ion currents.
In periaqueductal gray (PAG) neuron, ion current activated by *Corydalis tuber* was significantly suppressed by *strychnine*, a glycine receptor antagonist.
* represents $P < 0.05$ compared to the control. CT: *Corydalis tuber*

ion currents in a concentration-dependent manner in PAG neuron (Fig. 1).

2. Effect of Strychnine on *Corydalis tuber*-Activated Ion Currents

In order to evaluate pharmacological properties of the ion currents activated by *Corydalis tuber*, the magnitude of ion current elicited by 1 mg/ml *Corydalis tuber* was used as the control value, and strychnine, a glycine receptor antagonist, at concentrations of 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4}M, was applied simultaneously with 1 mg/ml *Corydalis tuber*. The ion current induced by 1 mg/ml *Corydalis tuber* was not changed by 10^{-7} M strychnine application, and treatment with 10^{-6} M strychnine inhibited the ion current about 14.00 ± 7.74 % with no statistical significance. Strychnine at concentrations of 10^{-5} M and 10^{-4} M inhibited ion currents induced by 1 mg/ml *Corydalis tuber* about 24.75 ± 5.94 % (n=6, p<0.05) and 74.25 ± 6.98 % (n=6, p<0.05) with statistical significance. These results showed that ion current induced by 1 mg/ml *Corydalis tuber* was suppressed by strychnine in a concentration-dependent manner (Fig. 2).

3. Modulation of Corydalis tuber on Glycine-Induced Ion Currents

To investigate the modulation of *Corydalis tuber* on the glycine-induced ion currents, the magnitude of ion current elicited by 10^{-5} M glycine was used as the control value and 0.05 mg/ml, 0.1 mg/ml, 0.5 mg/ml, and 1 mg/ml *Corydalis tuber* were applied simultaneously with 10^{-5} M glycine, respectively. 0.05, 0.1 and 0.5 mg/ml *Corydalis tuber* suppressed glycine-induced ion currents about 3.24 ± 1.53 %, 14.83 ± 1.61 % (n=6, p<0.05) and 45.74 ± 1.52 % (n=6, p<0.05), respectively. In contrast, 1.0 mg/ml *Corydalis tuber* potentiated glycine-induced ion current about 1.82 ± 2.72 % (n=6, p<0.05). The most potent suppression

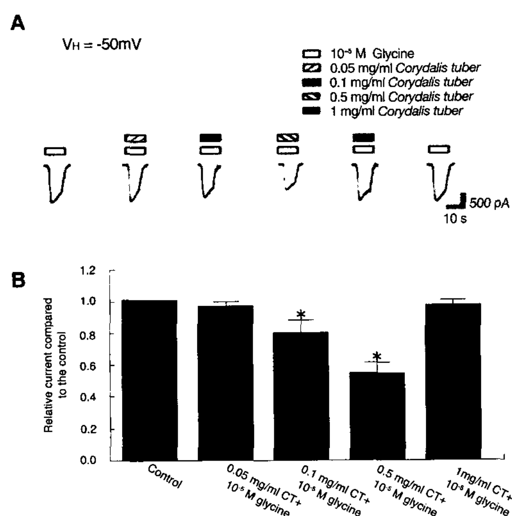

Fig. 3. Modulation of *Corydalis tuber* on glycine-activated ion currents.
Glycine-induced ion currents were significantly inhibited by 0.1mg/ml and 0.5mg/ml of *Corydalis tuber*.
* represents $P < 0.05$ compared to the control. CT: *Corydalis tuber.*

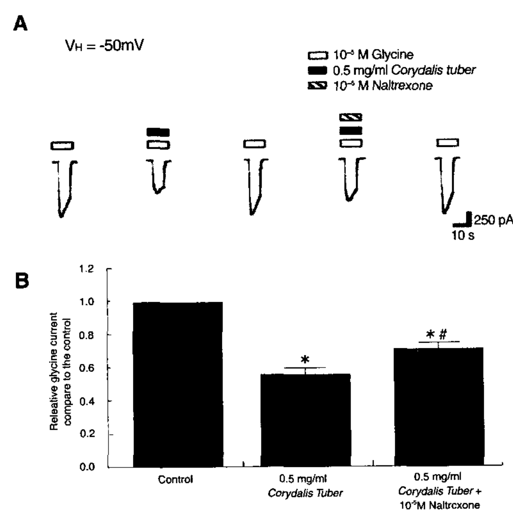

Fig. 4. Effect of naltrexone on *Corydalis tuber*-induced inhibition on glycine-activated ion current.
Inhibitory action of *Corydalis tuber* on glycine-activated chloride current was reduced by 10-5M naltrexone, an opioid antagonist and stable naloxone analogue.
* represents $P < 0.05$ compared to the control. CT: *Corydalis tuber.*

on the glycine-induced ion currents was observed at 0.5 mg/ml of *Corydalis tuber* (Fig. 3).

4. Effect of Naltrexone to *Corydalis tuber*-Induced Inhibition on Glycine-Activated Ion Current

To evaluate the involvement of opioid receptors on *Corydalis tuber*-induced inhibition on glycine-activated ion current in the PAG neurons, naltrexone, an opioid antagonist and stable naloxone analogue, was applied simultaneously with *Corydalis tuber*. In the present study, 0.5 mg/ml of *Corydalis tuber* inhibited glycine-induced ion current about 44.39 $\pm$ 4.65 % (n=6, $p<0.05$), while 10^{-5} M naltrexone significantly recovered glycine-induced ion current to 29.65 $\pm$ 4.77 % (n=6, $p<0.05$) (Fig. 4).

5. Effect of *N*-ethylmaleimide (NEM) to *Corydalis tuber*-Induced Inhibition on Glycine-Activated Ion Current

In order to elucidate the involvement of GTP-binding proteins (G-proteins) in *Corydalis tuber*-induced inhibition on glycine-activated ion current, the effect of NEM, a sulfhydryl alkylating agent, to the inhibition of *Corydalis tuber* on glycine-induced ion current was investigated. After perfusion with the standard solution containing NEM at a concentration of 5 x 10^{-5} M for 2 min, 0.5 mg/ml of *Corydalis tuber* inhibited glycine-induced chloride ion current about 44.39 $\pm$ 4.65 % (n=6, $p<0.05$) compared to control, but this inhibition decreased to 25.21 $\pm$ 9.82 % (n=6, $p<0.05$) after NEM perfusion (Fig. 5).

Discussion

Corydalis yanhusuo W. T. Wang, as a member of the

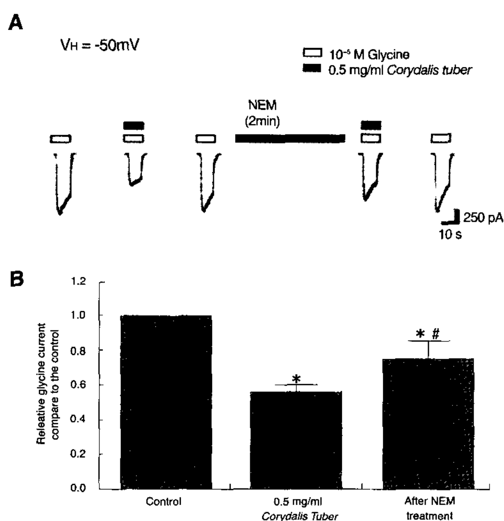

Fig. 5. Effect of N-ethylmaleimide (NEM) on *Corydalis tuber*-induced inhibition on glycine-activated ion current.
Inhibitory effect of Corydalis tuber on glycine-induced chloride current was significantly reduced by pretreatment with NEM, a sulfhydryl alkylating agent which selectively inhibits pertussis toxin (PTX)-sensitive G-proteins.
* represents $P < 0.05$ compared to the control. CT: *Corydalis tuber*.

poppy family, is closely related to the opium poppy. Corydalis has been used for centuries in Oriental medicine to invigorate the blood. A bitter herb with warming energy, the *Corydalis tuber* is known to help with the movement of blood, tonifying the blood itself and invigorating the vascular channels. It provides relief for conditions of blood deficiency such as dysmenorrhea, irregular menstruation, or amenorrhea with abdominal cramping. It is also indicated for conditions of blood stasis where the blood is not moving adequately. It has been shown to have a muscle relaxing effect, working on smooth muscles as well as skeletal muscles. It reduces coronary resistance, increases coronary blood flow, and dilates coronary blood vessels[1-4].

Because of the poppy family alkaloids, Corydalis is often used for its analgesic and sedative effects. The tuber contains more than 20 active alkaloids, the most active of which are the cordalines, THP and protopine. THP is analgesic and sedative and has been shown to work, at least in part, by blocking postsynaptic dopamine receptors in the central nervous system[5,6].

In this study, high concentrations of *Corydalis tuber* elicited ion current in the PAG neurons in a concentration-dependent manner (Fig. 1), and *Corydalis tuber*-induced ion current was inhibited by application of strychnine, glycine receptor antagonist (Fig. 2).

From this, it can be suggested that high concentrations (3 mg/ml and 5 mg/ml) of *Corydalis tuber* activate glycine receptors in the PAG neurons.

However, the major finding of this study is that low concentrations (0.1 mg/ml and 0.5 mg/ml) of *Corydalis tuber* reduce the glycine-induced ion current in the PAG neurons. Antagonists on glycine receptors as well as those of γ-aminobutyric acid (GABA) receptors are known to induce convulsion due to suppression on the inhibitory pathways in the CNS[19]. It has been proposed that the analgesic effect of opioids on the PAG works by suppressing the inhibitory influence of the neurotransmitters on the

neurons that form part of a descending antinociceptive pathway[8,12]. Based on the present results, it is possible that the inhibitory action of *Corydalis tuber* on glycine-induced ion current may activate the descending pain control system.

Naltrexone and naloxone are clinically prescribed as opioid antagonists and cross the blood-brain barrier. These non-selective opioid receptor antagonists block both central analgesia and adverse effects of opioid medications[20,21]. Stimulation on the PAG matter produces a kind of analgesia which is mediated by the release of endogenous opioids and blocked by pretreatment with naloxone[22]. In this study, naltrexone application reduced *Corydalis tuber*-induced inhibition on glycine-activated ion current in the PAG neurons. These results show that the opioid receptors are partly

involved in the inhibitory action of *Corydalis tuber* on glycine-activated ion current in the PAG neurons, suggesting that some components of *Corydalis tuber* exert analgesic action through opioid receptors in the PAG neurons. However, the inhibitory action of *Corydalis tuber* on glycine-activated ion current was not diminished completely by naltrexone application, suggesting that other components of *Corydalis tuber* may induce analgesic action through direct modulation on glycine-receptors in the PAG neurons.

Neurotransmitters acting through G-protein-coupled receptors change the electrical excitability of neurons. Activation of receptors can affect the voltage dependence, the speed of gating, and probability of opening of various ion channels, thus changing the computational state and outputs of a neuron. The G-proteins under consideration are heterotrimetric molecules with α-, β-, and γ-subunits. The α-subunits can be classified into three families, depending on whether they are targets for pertussis toxin (PTX), cholera toxin, or neither. In neurons, the most widespread modulatory signaling pathway is characterized by sensitivity to PTX, which indicates that the receptors couple to G-proteins of the Gi family, such as Gi or Go[23].

NEM has been used to block PTX-sensitive G-protein action. It is a sulfhydryl alkylating agent that can selectively inhibit PTX-sensitive G-protein mediated effects in the central[14,24,25], peripheral[26], and invertebrate neurons[27]. The advantage of using NEM is that it allows us to examine PTX-sensitive G-protein mediated action before and after inhibition of PTX-sensitive G-protein within a given recording. In this study, the inhibitory action of Corydlais tuber on glycine-induced ion current was reduced by NEM pretreatment for 2 min (Fig. 5). These results show that G-proteins are partly implicated in the inhibitory action of *Corydalis tuber* on glycine-activated ion current in the PAG neurons, suggesting that some components of *Corydalis tuber* exert analgesic action through G-proteins in the PAG neurons. However, the inhibitory action of *Corydalis tuber* on glycine-activated ion current was not diminished completely by NEM pretreatment. This means that some components of *Corydalis tuber* may induce analgesic action without involving G-proteins in the PAG neurons, suggesting directly affect glycine-receptors in the PAG neurons.

In Oriental medicine, aqueous extract from *Corydalis tuber* has been widely used for pain control. The PAG region of the brain is known to be involved heavily with nociception. These results suggest that inhibitory modulation of *Corydalis tuber* on glycine-activated ion current may activate the descending pain control system in PAG neurons, and this mechanism accounts for one of the analgesic actions of *Corydalis tuber*.

Conclusion

Corydalis tuber has been used in Oriental medicine for its analgesic and sedative effects.

The modulatory effect of *Corydalis tuber* on glycine-activated ion current in the acutely dissociated rat periaqueductal gray (PAG) neurons was investigated using the nystatin-perforated patch-clamp technique. High concentrations of Corydalis tuberelicited ion current, which was suppressed by strychnine application. Low concentrations of *Corydalis tuber* inhibited glycine-induced ion currents in the PAG neurons. Inhibitory action of *Corydalis tuber* on glycine-activated ion currents was reduced by naltrexone application. Application of N-methylmalemide (NEM), a sulfhydryl alkylating agent, also reduced the inhibitory action of *Corydalis tuber* on glycine-activated ion currents in the PAG neurons. These results suggest that the inhibitory effect of *Corydalis tuber* on glycine-activated ion current in the

PAG neurons is one of the analgesic mechanisms of *Corydalis tuber*.

References

1. Ahn Hyun-Seok, Park Young-Bae, Kang Sung-Keel. Effects of the *Corydalis tuber* aqua-acupuncture on the pain and convulsion in mice and gastric ulcer in rats. *The J. of Korean Acupuncture & Moxibustion society.* 1994;11(1):99-109.
2. Ahn Chun-Jae, Ahn Byoung-Choul, Park Dong-Seok. The analgesic effect of aqua-acupuncture with *Corydalis tuber*, Olibanum Mastix & Myrrha. *The J. of Korean Acupuncture & Moxibustion society.* 1994; 11(1):145-158.
3. Sung Il-Whan, Chae Woo-Seok. The effects of aqua-acupuncture of Hyunhoshag and Dangghuimi on thrombosis induced by endotoxin in rats. *The J. of Korean Acupuncture & Moxibustion society.* 1994; 11(1):391-403.
4. Ma W. G., Fukushi Y., Tahara S., Osawa T. Fungitoxic alkaloids from Hokkaido Papaveraceae. *Fitoterapia.* 2000;71:527-534.
5. Sagare A. P., Lee Y. L., Lin T. C., Chen C. C., Tsay H. S. Cytokinin-induced somatic embryogenesis and plant regeneration in *Corydalis yanhusuo* (Fumariaceae) - a medicinal plant. *Plant Sci.* 2000;160:139-147.
6. Lin M. T., Wang J. J., Young M. S. The protective effect of dl-tetrahydropalmatine against the development of amygdala kindling seizures in rats. *Neurosci Let.* 2002;320:113-116.
7. Fields, H. L., Heinricher M. M., Mason P. Neurotransmitters in nociceptive modulatory circuits. Ann. Rev. *Neurosci.* 1991;14:219-245.
8. Basbaum A. I., Fields H. L. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Ann. Rev. Neurosci.* 1984;7:309-338.
9. Mansour A., Fox C. A., Akil H., Watson S. J. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci.* 1995; 18:22-29.
10. Mason P. Central mechanisms of pain modulation. *Curr. Opin. Neurobiol.* 1999;9:436-441.
11. Duggan A. W. Electrophysiology of opioid peptides and sensory systems. *Br. Med. Bull.* 1983;39:65-70.
12. Renno W. M., Mullett M. A., Beitz A. J. Systemic morphine reduces GABA release in the lateral but not the medial portion of the midbrain periaqueductal gray of the rat. *Brain Res.* 1992;594:221-232.
13. Yaksh T. L., Jessell T. M., Gamse R., Mudge A. W., Leeman S. E. Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. *Nature.* 1980;286:155-157.
14. Han S. H., Cho Y. W., Kim C. J., Min B. I., Rhee J. S., Akaike N. μ-opioid agonist-induced activation of G-protein-coupled inwardly rectifying potassium current in rat periaqueductal gray neurons. *Neuroscience,* 1999;90:209-219.
15. Madison D., Nicoll R. A., Enkephalin hyperpolarizes interneurons in the rat hippocampus. *J. Physiol. (London),* 1988;398:122-130.
16. Kim, C. J., Rhee J. S., Akaike N. Modulation of high-voltage activated Ca^{2+} channels in the rat periaqueductal gray neurons by μ-type opioid agonist. *J. Neurophysiol.* 1997;77:1418-1424.
17. Rhim, H., Miller R. J. Opioid receptors modulate diverse types of calcium channels in the nucleus tractus solitarius of the rat. *J. Neurosci.* 1994;14: 7608-7615.
18. Gurwitz D. Profile. *Drug Discov.* Today. 2001;6:1178-1179.
19. Millan M. J. Descending control of pain. *Prog. Neurobiol.* 2002;66:355-474.
20. Kim Y., Shin M., Chung J., Kim E., Koo G., Lee C., Kim C. Modulation of *Chelidonii herba* on GABA activated chloride current in rat PAG neurons. *Am. J. Chin. Med.* 2001;29:265-279.
21. Crain S. M., Shen K. F. Acute thermal hyperalgesia elicited by low-dose morphine in normal mice is blocked by ultra-low-dose naltrexone, unmasking potent opioid analgesia. *Brain Res.* 2001;888:75-82.
22. Cannon J. T., Prieto G. J., Lee A., Liebeskind J. C. Evidence for opioid and non-opioid forms of stimulation-produced analgesia in the rat. *Brain Res.* 1982;243:315-321.
23. Hille B. Modulation of ion-channel function by G-protein-coupled receptors. *Trends Neurosci.* 1994;17: 531-536.

24. Morishita W., Kirov S. A., Pitler T. A., Martin L. A., Lenz R. A., Alger B. E. N-ethylmaleimide blocks depolarization-induced suppression of inhibition and enhances GABA release in the rat hippocampal slice in vitro. *J. Neurosci.* 1997;17:941-950.
25. Tang K. C., Lovinger D. M. Role of pertussis toxin-sensitive G-proteins in synaptic transmission and plasticity at corticostriatal synapses. *J. Neurophysiol.* 2000;83:60-69.
26. Shapiro M. S., Wollmuth L. P., Hille B. Modulation of Ca^{2+} channels by PTX-sensitive G-proteins is blocked by N-ethylmaleimide in rat synpathetic neurons. *J. Neurosci.* 1994;14:7109-7116.
27. Fryer M. W. An N-ethylmaleimide-sensitive G-protein modulates Aplysia Ca^{2+} channels. *Neurosci. Let.* 1992;146:84-86.