Effects of DX-9386, a Traditional Chinese Medicinal Prescription, on Long-Term Potentiation in the Dentate Gyrus in Rats

Yongxiang ZHANG, Hiroshi SAITO, Nobuyoshi NISHIYAMA,* and Kazuho ABE

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. Received April 15, 1994; accepted June 13, 1994

DX-9386, a traditional Chinese medicinal prescription consisting of ginseng, polygala, acorus and hoelen in the ratio of 1 : 1 : 25 : 50 (dry weight), was studied regarding the formation of long-term potentiation (LTP) in the dentate gyrus of anesthetized rats. Single oral administration of DX-9386 did not affect LTP formation evoked by suprathreshold tetanic stimulation; however, it significantly intensified the spike amplitude evoked by a subthreshold stimulation. LTP formation induced by suprathreshold tetanus was significantly inhibited by ethanol given either orally or intracerebroventricularly. DX-9386 significantly antagonized this inhibitory effect of ethanol. Basal spike amplitude was not influenced by DX-9386. These results indicate that DX-9386 potentiated LTP formation in the hippocampus and suggest that the ameliorative effect of this prescription on learning deficit model animals was, at least partly, due to its direct action on the hippocampus.

Keywords DX-9386; rat; dentate gyrus; LTP; ethanol

DX-9386, a traditional Chinese medicinal prescription consisting of ginseng, polygala, acorus and hoelen in the ratio of 1 : 1 : 25 : 50 (dry weight), has long been employed clinically to treat mental diseases in China.¹⁾ Neuropharmacological studies of this traditional prescription have been performed in our laboratory, which revealed that DX-9386 ameliorated the impairment of learning and memory ability induced by the oral administration of ethanol (unpublished data) or by brain lesion²⁾ in mice. Chronic ingestion of DX-9386 in the diet improved learning behaviors in the senescence accelerated mouse.³⁾ These results suggested that this prescription might enhance the cognitive function of the central nervous system.

Long-term potentiation (LTP) of an evoked response in the hippocampus is one of the manifestations of synaptic plasticity and is considered to be the cellular basis of the learning and memory process.⁴⁻⁶⁾ Synaptic plasticity in the hippocampus showed a good correlation with the performance in shuttle box test in rats.⁷⁾ Rats with an inborn inferior performance required a greater threshold to generate LTP in comparison to rats with an inborn superior performance in shuttle box test.⁸⁾ Thus, it could be speculated that a drug which influences learning and memory might also affect the processes of LTP generation in the hippocampus. Ethanol has been known to impair the learning and memory ability in human^{9,10)} and rodents.¹¹⁾ Moreover, a low concentration of ethanol inhibited LTP generation in a rat hippocampal slice *in vitro*,¹²⁾ or in the dentate gyrus of anesthetized rats when given by *p.o.* or *i.c.v.* *in vivo*.¹³⁾ These lines of evidence also indicate the close relationship between learning and LTP.

To elucidate the mechanisms of the central effect of DX-9386, we studied the effect of the prescription on LTP generation in the hippocampal dentate gyrus of anesthetized rats.

MATERIALS AND METHODS

The experiment was performed according to the method employed in our laboratory.^{14,15)} Male Wistar rats, 8—10 weeks old, were anesthetized by *i.p.* injection of a mixture of urethane and α -chloralose (1g/kg and 25mg/kg, respectively) and fixed in a stereotaxic frame. A bipolar stainless-steel electrode with a tip separation of 0.8mm was inserted into the left entorhinal cortex (8.1mm posterior to bregma, 4.4mm lateral to midline and approximately 3.0mm below the dura) to stimulate the perforant path. A monopolar recording electrode was placed in the granule cell layer of the ipsilateral dentate gyrus (3.5mm posterior to bregma, 2.0mm lateral to midline and about 3.0mm below the dura). The depths of both stimulating and recording electrodes were adjusted to obtain a desired response. A single test stimulus (0.08 ms duration) was applied at a constant interval of 30 s and the evoked field potential was recorded extracellularly. The stimulus intensity was set at a level of 50% of the population spike of the maximum amplitude. A brief suprathreshold (30 pulses, 60 Hz) or subthreshold (20 pulses, 60 Hz) tetanic stimulation was applied at the same stimulus intensity to evoke LTP after the response became stable. The evoked field potential was recorded for 60 min after the tetanus. LTP was considered to occur when the tetanus-potentiated spike amplitude was maintained at a level of 20% higher than the baseline and lasted for more than 30 min.^{14,15)}

DX-9386 (Daiichi Pharmaceutical Co., Ltd.) was dissolved in saline and given to rats in a single dose of 500 mg/kg, *p.o.* 30 min before tetanus. Ethanol (30% in saline) was given by either *p.o.* administration (2 ml/kg) or *i.c.v.* injection (5 μ l) through a canula inserted into the stomach or the right lateral cerebral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to midline and about 4.2 mm below the dura) 20 or 15 min before tetanus, respectively.

RESULTS

Effect of DX-9386 on LTP Formation Evoked by Suprathreshold Tetanus Suprathreshold tetanus (60 Hz, 30 pulses) induced LTP in the control rats. A single oral administration of DX-9386 (500 mg/kg) 30 min prior to the tetanus did not significantly influence the LTP induced by tetanic stimulation (Fig. 1).

Effect of DX-9386 on LTP Formation Evoked by Subthreshold Tetanus LTP was not evoked in the control group when a subthreshold tetanic stimulation (20 pulses, 60 Hz) was applied (Fig. 2). However, DX-9386 treatment significantly increased the population spike amplitude

Fig. 1. Effect of DX-9386 on LTP Formation Induced by Suprathreshold Tetanic Stimulation in Rat Dentate Gyrus

A: typical records of evoked potentials in the dentate gyrus before (left) or after (right) a tetanus (60 Hz, 30 pulses). The amplitude of a population spike was defined as the average of the amplitude from the first positive peak (1) to the succeeding negative peak (2) and the amplitude from the negative peak (2) to the second positive peak (3). Calibration bars: vertical 2 mV, horizontal 10 ms. B: potentiation of a population spike induced by suprathreshold tetanic stimulation in the control group ($\circ$, $n=5$) and in the DX-9386 treated group ($\bullet$, $n=6$). The ordinates indicate spike amplitude expressed as a percentage of baseline values at 0 min (immediately before a tetanic stimulation). Data are represented as mean $\pm$ S.E.M. of n observations.

Fig. 3. Effect of DX-9386 on Ethanol-Inhibited LTP Formation Induced by Suprathreshold Tetanic Stimulation in Rat Dentate Gyrus

Thirty percent ethanol (2 ml/kg) was given by *p.o.* administration 20 min before a tetanus (A) or by *i.c.v.* injection (5 μ l/rat) 15 min before a tetanus (B). The ordinates indicate spike amplitude expressed as a percentage of baseline values at 0 min (immediately before a tetanic stimulation) in the control group ($\circ$, $n=6$), the ethanol treated group (∇ , $n=6-10$) and the ethanol plus DX-9386 group ($\bullet$, $n=7-10$). Data are represented as mean $\pm$ S.E.M. of n observations. *a*) $p < 0.05$, *b*) $p < 0.01$ vs. ethanol treated group in ANOVA followed by Duncan's multiple range test.

evoked by the subthreshold tetanus for about 30 min. This potentiation by the prescription gradually decreased to the control level within 60 min (Fig. 2).

Effects of DX-9386 on Ethanol-Induced Inhibition of LTP Formation Evoked by Suprathreshold Tetanus LTP generation was significantly inhibited by the oral administration of ethanol (30%, 2 ml/kg) 20 min before a tetanus (Fig. 3A). DX-9386 antagonized the inhibitory effect of ethanol when it was given 10 min before ethanol administration, and the amplitude of evoked potential was significantly increased during the time period of 15–40 min after the tetanus compared to that of ethanol-treated group (Fig. 3A). Intracerebroventricular injection of ethanol (30%, 5 μ l) 15 min before a tetanus also strongly prevented the completion of LTP (Fig. 3B). DX-9386 treatment tended to elevate the amplitude of the evoked potential inhibited by ethanol, and significant potentiation was recorded at 40 min after the tetanus (Fig. 3B). However, the antagonizing efficacy of DX-9386 against *i.c.v.* injected ethanol was weaker than against *p.o.* administered ethanol.

Effect of DX-9386 on Basal Response The evoked potential was observed for 90 min following DX-9386 administration without a tetanic stimulation. As shown in Fig. 4, DX-9386 did not significantly influence the basal spike amplitude.

Fig. 2. Effect of DX-9386 on LTP Formation Induced by Subthreshold Tetanic Stimulation in Rat Dentate Gyrus

Subthreshold tetanus (60 Hz, 20 pulses) did not evoke LTP in the control group ($\circ$, $n=6$). The DX-9386 treated group showed a significantly larger spike amplitude than the control group for 30 min after the tetanic stimulation ($\bullet$, $n=6$). The ordinates indicate spike amplitude expressed as a percentage of baseline values at 0 min (immediately before a tetanic stimulation). Data are represented as mean $\pm$ S.E.M. of n observations. *a*) $p < 0.05$ vs. control group in Student's *t*-test.

Fig. 4. Effect of DX-9386 on Basal Population Spike Induced by a Testing Stimulus without the Application of a Tetanus

DX-9386 was administered at 0 min and the basal response was observed up to 90 min. Data are represented as mean $\pm$ S.E.M. of n observations ($n=4$).

DISCUSSION

Our previous studies revealed that DX-9386 ameliorated the learning and memory deficiency in several animal models.^{2,3} The pharmacological mechanism, however, remains largely unknown. The present study demonstrated that a single oral administration of DX-9386 potentiated the LTP generation induced by a subthreshold tetanic stimulation and antagonized the inhibitory effect of ethanol on LTP generation in the rat dentate gyrus, suggesting that DX-9386 was involved in the process of LTP formation in the hippocampus.

DX-9386 potentiated the population spike amplitude induced by a subthreshold tetanus, but this potentiation went down to the control level within 60 min. It has been presumed that LTP consists of several phases, *i.e.* induction phase, early maintenance phase and late maintenance phase, which are mediated by different mechanisms.¹⁶ The potentiation which returns to the base line level within 30 min is regarded as short-term potentiation (STP), and STP is coupled with the induction of LTP.¹⁶ DX-9386 enhanced the STP, presumably by affecting the induction and/or early maintenance phase of LTP. The prescription might have lowered the threshold or enhanced the stimulating efficacy of a subthreshold tetanus. This STP-promoting effect of DX-9386 may correlate with the ameliorative effect on learning of the prescription. Meanwhile, DX-9386 did not influence LTP formation induced by a suprathreshold tetanic stimulation. Although the population spike amplitude after tetanus in the drug-treated group seemed to be lower than that in the control group during the initial 30 min, the spike amplitude maintained a level at around 150% above the baseline level for more than 60 min. No statistical difference was observed between the control and DX-9386 treated groups. These results indicated that DX-9386 enhanced the evoked potential stimulated by a subthreshold tetanus, but not suprathreshold tetanus, suggesting an adaptive role of the prescription on synaptic transmission. DX-9386 administration did not affect the basal spike amplitude, which ruled out the possibility that the basal synaptic transmission in the hippocampal dentate gyrus gradually increased after the drug application.

Ethanol-induced impairment of learning and memory may be partially due to its inhibitory effects on LTP

generation.¹³ The induction of LTP has been considered to be mediated by a *N*-methyl-D-aspartate (NMDA) receptor,⁵ and ethanol was supposed to block this NMDA receptor to inhibit LTP formation in the hippocampus.^{11,17} In our previous study, DX-9386, at a dose of 500 mg/kg (*p.o.*), ameliorated the learning behaviors of mice impaired by an oral administration of 30% or 40% ethanol (unpublished observation). In this study, DX-9386 at the same dose showed an obvious facilitative effect on LTP formation inhibited by ethanol administration, suggesting that DX-9386 may be involved in the interactions between ethanol and the NMDA receptor in the hippocampus. The difference in antagonizing efficacy of DX-9386 against ethanol given *p.o.* or *i.c.v.* suggested that some other influences may be involved, such as an alternation of the pharmacokinetic process, because ginseng was reported to accelerate blood alcohol clearance in human.¹⁸

Senescence is a major reason for learning and memory deficiency manifestation. An imbalance in the neuroendocrine immunomodulation (NIM) network is closely related to systematic aging.^{19–24} Therefore, a drug which modulates immune and endocrine functions may affect the NIM network to influence the cognitive function. Ginseng, a component of DX-9386, potentiates immune function^{25–27} and modulates endocrine balance,^{28,29} in addition to its central effects.^{13,28,30–32} An extract of hoelen, another component of DX-9386, is also known to modulate immune and endocrine functions.^{33–35} However, our previous study revealed that chronic administration of the prescription did not potentiate the immune response in senescence accelerated mice.³⁶ This result suggests that the “improving effect” of the preparation on learning and memory may be achieved by a direct central effect rather than a modulative effect on the NIM network. The central effect of DX-9386 may, at least partially, contribute to its modulative effect on synaptic plasticity in the hippocampus. Further studies to elucidate the mechanism of the central effect of this prescription are now being conducted in our laboratory.

REFERENCES

- 1) “Dictionary of Traditional Chinese Medicine (Zhong Yao Da Zi Dian),” Vol. 2, ed. by Suzhou New Medical College, Shanghai People’s Publication House, Shanghai, 1977, p. 612.
- 2) Y. P. Zhou, N. Nishiyama, H. Saito, Abstracts of Papers, The 112th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 1992, part 3, p. 12.
- 3) Y. P. Zhou, N. Nishiyama, H. Saito, Abstracts of papers, The 113th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, March 1993, part 3, p. 34.
- 4) T. J. Teyler, P. DiScenna, *Annu. Rev. Neurosci.*, **10**, 131 (1987).
- 5) T. V. P. Bliss, G. L. Collingridge, *Nature* (London), **361**, 31 (1993).
- 6) G. Lynch, U. Staubli, *Brain Res. Rev.*, **16**, 204 (1991).
- 7) O. A. Ramirez, H. F. Carrer, *Neurosci. Lett.*, **104**, 152 (1989).
- 8) O. A. Ramirez, O. A. Orsingher, H. F. Carrer, *Neurosci. Lett.*, **92**, 275 (1988).
- 9) N. K. Mello, “The Biology of Alcoholism,” Vol. 2, ed. by B. Kissin, H. Begleiter, Plenum, New York, 1972, pp. 219–291.
- 10) J. M. Ritchie, “The Pharmacological Basis of Therapeutics,” ed. by A. G. Gillman, L. S. Goodman, T. W. Rall, F. Murad, McMillan, New York, 1985, pp. 372–386.
- 11) H. Saito, “Recent Advances in Ginseng Studies,” ed. by S. Shibata, Y. Ohtsuka, H. Saito, Hirokawa, Tokyo, 1990, pp. 99–111.

- 12) R. D. Blitzer, O. Gil, E. M. Landau, *Brain Res.*, **537**, 203 (1990).
- 13) M. Sugiura, H. Saito, K. Abe, Y. Shoyama, *Phytother. Res.*, in press.
- 14) N. Chida, H. Saito, K. Abe, *Brain Res.*, **593**, 57 (1992).
- 15) A. Mizutani, H. Saito, K. Abe, *Brain Res.*, **605**, 309 (1993).
- 16) K. Abe, F.J. Xie, H. Saito, *Brain Res.*, **547**, 171 (1991).
- 17) D. M. Lovinger, G. White, F. F. Weight, *Science*, **243**, 1721 (1989).
- 18) F. C. Lee, J. H. Ko, J. K. Park, J. S. Lee, *Clin. Exp. Pharmacol. Physiol.*, **14**, 543 (1987).
- 19) N. Fabris, L. Piantanelli, "Hormones and Aging," ed. by R. C. Adelman, G. S. Roth, CRC Press, Boca Raton, 1982, pp. 167—181.
- 20) N. Fabris, "Immunoregulation in Aging," ed. by A. Facchini, J. J. Haaijman, G. Labo, Eurage, Rijswijk, The Netherlands, 1986, pp. 117—730.
- 21) R. G. Goya, S. Takahashi, K. L. Quigley, Y. E. Sosa, A. L. Goldstein, J. Meites, *Mech. Aging Dev.*, **41**, 219 (1987).
- 22) R. G. Goya, Y. E. Sosa, K. L. Quigkey, P. E. Gottschall, A. L. Goldstein, J. Meites, *Neuroendocrinology*, **47**, 379 (1988).
- 23) R. G. Goya, K. L. Quigley, S. Takahashi, R. Reichhart, J. Meites, *Mech. Aging Dev.*, **49**, 119 (1989).
- 24) R. G. Goya, *Gerontology* (Basel), **37**, 298 (1991).
- 25) B. Kenarova, H. Neychev, C. Hadjiivanova, V. D. Petkov, *Jpn. J. Pharmacol.*, **54**, 477 (1990).
- 26) J. Y. Kim, D. R. Germolec, M. I. Luster, *Immunopharmacol. Immunotoxicol.*, **12**, 257 (1990).
- 27) F. Scaglione, F. Ferrara, S. Dugnani, M. Falchi, G. Santoro, F. Fraschini, *Drugs Exp. Clin. Res.*, **16**, 537 (1990).
- 28) V. Petkov, "Recent Advances in Ginseng Studies," ed. by S. Shibata, Y. Ohtsuka, H. Saito, Hirokawa, Tokyo, 1990, pp. 83—98.
- 29) A. A. Filaretov, T. S. Bogdanova, T. T. Podvigina, A. I. Bodganov, *Exp. Clin. Endocrinol.*, **92**, 129 (1988).
- 30) T. C. Ma, Q. H. Yu, M. H. Chen, *Acta Pharmacologica Sinica*, **12**, 403 (1991).
- 31) N. Nishiyama, "Recent Advances in Ginseng Studies," ed. by S. Shibata, Y. Ohtsuka, H. Saito, Hirokawa, Tokyo, 1990, pp. 125—137.
- 32) C. G. Benishin, "Recent Advances in Ginseng Studies," ed. by S. Shibata, Y. Ohtsuka, H. Saito, Hirokawa, Tokyo, 1990, pp. 139—143.
- 33) J. Tseng, J. G. Chang, *Chung Hua Min Kuo Wei Sheng Wu Chi Mien I Hsueh Tsa Chih*, **25**, 1 (1992).
- 34) S. Usuki, *Am. J. Chin. Med.*, **16**, 11 (1988).
- 35) S. Usuki, *Am. J. Chin. Med.*, **16**, 107 (1988).
- 36) Y. Zhang, K. Takashina, H. Saito, N. Nishiyama, *Biol. Pharm. Bull.*, **17**, 866 (1994).