Hoelen (Poria cocos Wolf) and Ginseng (Panax Ginseng C. A. Meyer), the Ingredients of a Chinese Prescription DX-9386, Individually Promote Hippocampal Long-Term Potentiation in Vivo

Miroslav Smriga, Hiroshi Saito, and Nobuyoshi Nishiyama*

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. Received November 11, 1994; accepted December 12, 1994

DX-9386 is a traditional Chinese medicinal prescription consisting of ginseng (Panax Ginseng C. A. Meyer), polygonia (Polygonia Temuifolia Willdenew), acorus (Acorus Gramineus Soland), and hoelen (Poria Cocos Wolf). We recently found that oral administration of the prescription at a dose of 500 mg/kg intensified the formation of long-term potentiation (LTP) in the dentate gyrus of anesthetized rats. To evaluate the individual contribution of separate ingredients in DX-9386 towards the observed biological activity, we investigated their direct influence on LTP formation in vivo. A single oral administration of hoelen and ginseng (250 and 500 mg/kg) significantly increased the spike amplitude evoked by a subthreshold tetanic stimulation at time intervals up to 30 min after tetanus. Only minor effects of polygonia (500 mg/kg) and no influence of acorus up to 500 mg/kg were observed. No drugs affected the basal spike amplitude induced by a test stimulus. In addition, we ascertained that DX-9386 was also active at a dose of 250 mg/kg. Taken together, these results indicate that hoelen and ginseng are the active components of DX-9386 with regard to the enhancement of hippocampal LTP.

Key words: DX-9386; hoelen; ginseng; LTP; dentate gyrus

The long-time use of DX-9386, a traditional Chinese medicinal prescription, in the treatment of mental disorders and aging-related diseases within the Chinese population was the starting point in an attempt to find out whether, and in case of a positive answer, how the action of the prescription is linked to functions of the central nervous system (CNS). DX-9386 consists of ginseng (Panax Ginseng C. A. Meyer), polygonia (Polygonia Temuifolia Willdenew), acorus (Acorus Gramineus Soland), and hoelen (Poria Cocos Wolf) in the ratio of 1:1:25:50 (dry weight). In our previous experiments the prescription ameliorated the learning and memory deficiency in several animal models. The prescription improved the deteriorated learning performances of mice after amygdala-lesion, ethanol administration, and thymectomy. A direct effect of DX-9386 on central functions, together with alternative mechanisms such as influence on the neuroendocrine immunomodulation network were considered. However, unlike ginseng, DX-9386 did not improve the immune response reduced by thymectomy. In addition, Zhang et al. showed that DX-9386 orally administered at a dose of 500 mg/kg enhanced long-term potentiation (LTP) in the dentate gyrus of anesthetized rats and suppressed the inhibitory effect of ethanol on the LTP generation in the same hippocampal structure. Long-term potentiation of synaptic transmission in the hippocampus is nowadays the primary experimental model for investigating the synaptic basis of learning and memory in vertebrates (for review see Ref. 12). In the hippocampal area there are at least two distinct mechanisms by which LTP is produced at different sites. At synaptic input from the mossy fibers onto pyramidal neurons in the area CA2, LTP can be induced solely by high-frequency stimulation of the presynaptic terminal. The strength of the stimulation required is not influenced by depolarization of the postsynaptic membrane. Since this type of LTP is independent of events at neighboring synapses, it is called nonassociative. A second type of LTP occurs at the synapses onto dentate gyrus and CA1 regions. In both regions temporally pairing activity in a "weak" synaptic input (incapable of generating LTP by itself) with activation of a strong input results in LTP of the weak input. This associative property can be considered a cellular analog of associative learning. Regarding this fact and the ease with which LTP can be induced in the hippocampal dentate gyrus following tetanization of the perforant path in the anesthetized rats, we have chosen this area for the determination of a drug's effects.

Since neither minimal active dose nor ingredient(s) of the prescription responsible for the detected biological activities were known in the present study, we concentrated our attention on these questions, testing various doses of DX-9386 as well as its ingredients. Our aim was to elucidate whether hoelen, ginseng, polygonia or acorus alone is able to mimic the profound ability of DX-9386 to potentiate LTP formation induced by a subthreshold tetanic stimulation in the hippocampal dentate gyrus of anesthetized rats.

MATERIALS AND METHODS

Male Wistar rats (SLC, Japan) 7 to 9 weeks old were anesthetized with a combination of urethane (1 g/kg, i.p.) and x-chloralose (25 mg/kg, i.p.). A bipolar stainless stimulating electrode (0.25 mm in diameter) with 0.8 mm tip separation was placed in the left entorhinal cortex (8.1 mm posterior to bregma, 4.4 mm lateral to midline, approximately 3.0 mm ventral to dura, with bregma and lambda in the same horizontal plane) to stimulate the medial perforant path. A monopolar stainless recording electrode (0.25 mm in diameter) was placed in the granule cell layer of the ipsilateral dentate gyrus (3.5 mm posterior to bregma, 2.0 mm lateral to midline, approximately

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3.0 mm ventral to dura). The depth of the electrodes was adjusted to produce a maximum response. A single test stimulus (0.08 ms duration) was applied at intervals of 30 s and the evoked field potential was recorded extracellularly. Stimulus intensity was set to a level which produced a population spike of about 50% of the maximum amplitude. After the response became stable, drugs were administered. The drugs dissolved in saline were delivered orally via a plastic tube inserted directly to the animals stomach. A volume of 1 ml of each tested substance was applied, reaching the required final concentrations. DX-9386, together with water extracts of hoelen, ginseng, polygala and ethanol extract of acorus were individually prepared and supplied by Daichi Pharmaceutical Co. Ltd. (Tokyo, Japan). To induce LTP, tetanic stimulation was applied at the same stimulus intensity through the same system of electrodes as used for test stimulation 30 min after the administration of a drug. A tetanic stimulation consisting of 20 pulses at 60 Hz was used to generate potentiation of the evoked potential. In terms of magnitude, the potentiation observed in a saline treated animal was characterized by only a transient increase in the spike amplitude, which could not be characterized as LTP; that is, it is N-methyl-D-aspartate receptor-dependent and lasts for more than 1 h.\textsuperscript{12} LTP in the hippocampal dentate gyrus was considered to have occurred if the potentiated spike amplitude remained at a level more than 20% above the baseline level for 30–60 min after tetanic stimulation. In terms of LTP induction frequency, tetanic stimulation of 20 pulses at 60 Hz induced LTP in only 1 out of 5 cases. Hence, the described tetanus was regarded as a subthreshold stimulation in inducing LTP. Results obtained from all rats regardless of LTP induction were included in the data calculation. Experiments were done at room temperature.

Under these experimental conditions, the changes in population spike amplitude were regarded as a responsible and the most easily accessible measure of cellular processes, since each change in signal transduction is finally reflected in the generation of the action potential and in alterations of its firing rate. As shown in Fig. 1A, the spike amplitude was defined as the average of the amplitude from the first positive peak (1) after the rising phase of the evoked potential, to the succeeding geometrical minimum (2) after downward deflection, and the amplitude from the minimum (2) to the second positive peak (3).

The effects of the tested drugs were assessed as percentage difference between the basal and tetanically stimulated levels. The results were expressed as means ± S.E.M. of 5 rats. The data were statistically evaluated using two-tail paired \( t \)-test.

RESULTS

In the first part of the experimental work, the effect of DX-9386 was assessed. Since Zhang et al.,\textsuperscript{11} showed that DX-9386, orally administered, did not affect the basal evoked potential in the dentate gyrus, the experiments were concentrated directly on the effect of prescription upon LTP generation. As depicted in Fig. 1B, oral administration of DX-9386 at a dose of 250 mg/kg caused a significant increase in the magnitude of the spike amplitude induced by the subthreshold tetanus. On the other hand, the prescription applied at a dose of 125 mg/kg did not affect the magnitude of synaptic responses induced by the same tetanic stimulation.

Next, the possible effects of all individual components of DX-9386 alone on the basal spike amplitude and LTP generation evoked by the described subthreshold tetanus were tested. Water extract of hoelen administered orally.
(500 mg/kg) did not influence the synaptic potential evoked by low-frequency test stimulation up to 90 min after the drug treatment (Fig. 2). Similarly, oral administration of ginseng, polygala or aconis at a dose of 500 mg/kg did not cause any significant changes in the basal synaptic responses evoked by the test stimulation (n=4, data not shown). When the subthreshold tetanic stimulation was applied 30 min after oral administration of hoelen (250 and 500 mg/kg), the drug under study significantly increased the population spike amplitude in both doses used (Fig. 3B and C). No statistically significant effect upon the spike amplitude was observed when hoelen was applied at a dose of 125 mg/kg (Fig. 3A). Similarly, the water extract of ginseng, administered at doses of 250 and 500 mg/kg, but not 125 mg/kg, significantly augmented the spike amplitude, as shown in Fig. 4A, B and C. Both drugs caused a rapid increase in spike amplitude compared to the saline treated groups, the difference being statistically significant at time intervals up to 30 min after tetanus.

Contribution of the last two ingredients in DX-9386 to the resultant effect of the prescription upon LTP formation was also tested. Polygala (500 mg/kg), applied orally 30 min prior to subthreshold tetanus influenced the spike amplitude significantly only at two time intervals (Fig. 5B). No significant effect was observed after the dose was lowered to 250 mg/kg (Fig. 5A). The last component, aconis, did not show any ability to potentiate LTP formation in dentate gyrus of anesthetized rats even at 500 mg/kg (Fig. 6).

DISCUSSION

DX-9386 has recently been intensively studied with
the initial phase of LTP formation in the dentate gyrus of anesthetized rats was also reported.\textsuperscript{11} Consistent with the work of Zhang et al.,\textsuperscript{11} we confirmed the ability of DX-9386 to enhance LTP formation after its oral administration. In addition, we showed that the prescription is active even at a dose of 250 mg/kg, but DX-9386 lacks the potency to promote LTP formation at a dose of 125 mg/kg. In spite of the obvious danger in attempting to leap directly from cellular physiology to mechanisms of learning and memory, LTP in hippocampus is the dominant model of these processes. This assumption, together with the facts that DX-9386 at doses of 250 and 500 mg/kg positively influenced LTP formation during the first 30 min after tetanus and that the prescription ameliorated the memory acquisition deficits by a single or chronic administration at the comparable doses\textsuperscript{5,6}, suggested that the ameliorative effect of the prescription on the previously mentioned learning deficit animal models\textsuperscript{3–7} was, at least partly, due to its direct action in the hippocampus.

A basic question emerging at this stage of experimental knowledge concerned the extent of individual contribution of the components in DX-9386 toward the described results. We studied this question by testing the ability of each ingredient alone to stimulate LTP formation, keeping the experimental conditions unchanged. All four components are frequently prescribed in Chinese medicine due to their advantageous properties.\textsuperscript{13} Hoelen is regarded to be a potent diuretics and cardiotonics. The drug also lowers blood sugar levels and contributes to an increase in the immune response of the human body to cancer cells.\textsuperscript{2,13} Polygala stimulates bronchial secretion. It also offers sedative and tranquilizing effects, as well as some antibacterial properties.\textsuperscript{2} Acorus is a drug with valuable sedative and antibacterial features. Numerous beneficial central effects of ginseng are well established.\textsuperscript{14–17} Ginseng also potentiates immune and modulate endocrine functions.\textsuperscript{9,10,14,18} Since none of the tested drugs significantly influenced the synaptic potential evoked by the low-frequency test stimulation, we presumed that they did not affect the basal synaptic functions. When the subthreshold tetanus was applied 30 min after oral administration of hoelen or ginseng, both drugs showed a profound ability to stimulate LTP for about 30 min thereafter. From the extraction yield calculation, 500 mg of DX-9386 contains 39 mg of polygala, 42 mg of ginseng, 279 mg of hoelen and 140 mg of acorus. The active doses of hoelen, 250 and 500 mg/kg, and the time-course of altered spike amplitude induced by hoelen and ginseng were consistent with the effective doses and time-course of DX-9386. Therefore, we assumed that hoelen is the component predominantly responsible for the stimulatory effect of DX-9386 on LTP. Although ginseng alone cannot be responsible for the entire effect of DX-9386, it may act supportively to hoelen and/or work synergistically with hoelen in the LTP induction. Nevertheless, hoelen and ginseng are reported to be influential in several peripheral functions.\textsuperscript{9,10,14,18–21} Consequently, the influences of peripheral systems may have affected the synaptic transmission in the hippocampus, especially when detailed information on how orally administered hoelen or ginseng
is absorbed, metabolized and distributed throughout tissues is lacking.

In conclusion, these results confirmed the promotive effect of oral administration of DX-9386 (blend of hoelen, ginseng, polygala and acorus) on LTP formation and showed that its effect on the hippocampus is dose-dependent. In addition, two new plants, namely hoelen (Porta Cocos Wolf) and ginseng (Panax Ginseng C. A. Meyer) were found to be the potent stimulators of LTP formation in the dentate gyrus after their oral administration.

REFERENCES AND NOTES

1) On leave from: The Institute of Experimental Endocrinology, Bratislava, Slovakia.