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Effects of spinorphin and tynorphin on synaptic transmission in rat hippocampal slices

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Abstract

Spinorphin has been isolated from the bovine spinal cord as an endogenous inhibitor of enkephalin-degrading enzymes (aminopeptidase, dipeptidyl aminopeptidase III, angiotensin-converting enzyme and enkephalinase), and tynorphin has been synthesized as a more potent inhibitor of dipeptidyl aminopeptidase III. In this study, the effects of spinorphin and tynorphin on synaptic transmission were studied in rat isolated hippocampal slices. Field potentials were recorded from the CA1 region after stimulation of Schaffer collaterals. Spinorphin (1 μM), which alone had no effect, potentiated the facilitatory effects of enkephalin on the filed potentials at a stimulation interval of 15 s. At a stimulation interval of 10–4 s, spinorphin alone frequency dependently inhibited the field potential. On the other hand, tynorphin (1 μM), which alone had no effect at any stimulus interval, tended to potentiate the facilitatory effects of enkephalin. Spinorphin blocked long-term potentiation induced by tetanic stimulation (100 Hz, 1 s), whereas tynorphin had no effect on long-term potentiation. These results suggest that, at a low stimulation frequency, spinorphin potentiates the facilitatory effects of enkephalin by preventing degradation of enkephalin, whereas at a high stimulation frequency spinorphin use dependently inhibits synaptic transmission independently of enkephalin. On the other hand, tynorphin tends to potentiate the facilitatory effects of enkephalin without use-dependent inhibition.

Keywords: Spinorphin; Tynorphin; Enkephalin-degrading enzyme; Hippocampus; Field potential

1. Introduction

Enkephalin is an endogenous opioid peptide (Hughes et al., 1975). A genuine effect of enkephalin itself has not been observed because it is hydrolyzed easily by its degrading enzymes in the body (Hambrock et al., 1976) (Fig. 1). For this reason, synthetic substances, which inhibit enkephalin-degrading enzymes, have been used (Barclay and Phillippa, 1980; Dickenson, 1986). Spinorphin (Leu-Val-Val-Tyr-Pro-Trp-Thr:LVVYPWT) has been isolated from the bovine spinal cord as an endogenous inhibitor of enkephalin-degrading enzymes (Nishimura and Hazato, 1993). Spinorphin inhibits aminopeptidase, dipeptidyl aminopeptidase III, angiotensin-converting enzyme and enkephalinase. Spinorphin analogs have been synthesized and assayed for their inhibitory activity toward enkephalin-degrading enzymes in studies of the structure–activity relationship of spinorphin. Tynorphin (VVYPW), an N-terminal and C-terminal truncated form of spinorphin, has more potent specific inhibitory activity toward dipeptidyl aminopeptidase III (Yamamoto et al., 2000).

It has been reported that spinorphin has an antinociceptive effect (Nishimura et al., 1993), an inhibitory effect on electrically evoked contractions of mouse vas deferens and guinea pig ileum (Nishimura et al., 1993), and an anti-inflammatory effect (Yamamoto et al., 1997, 1998). It has been shown that tynorphin has a more potent antinociceptive effect than spinorphin (Ueda et al., 2000). There is much interest in the role of spinorphin and tynorphin in the body, but the mechanisms of their effects at nerve level have remained unclear.

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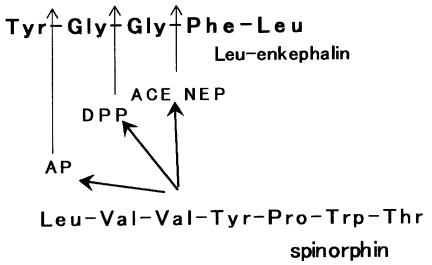

Fig. 1. Degradation of [Leu^5]enkephalin and inhibition of the degradation by spinorphin. AP: aminopeptidase, DPP: dipeptidyl aminopeptidase III, ACE: angiotensin-converting enzyme, NEP: enkephalinase.

In hippocampal slices, enkephalin potentiates synaptic responses in the CA1 region and the effect is antagonized by the opioid receptor antagonist naloxone (Haas and Ryall, 1980). The enkephalinase inhibitors, thiorphan and *N*-[L-(1-carboxy-2-phenyl)ethyl]-L-phenylalanyl-β-alanine (SCH 32615), potentiate the facilitatory effects of the enkephalin analogs, [D-Ala^2,*N*-methyl-Phe^4,Gly-ol^5]enkephalin (DAGO) and [D-Ala^2,Met^5]enkephalinamide (DAEAM), in the CA1 region (Proietti et al., 1991; Scotti et al., 1991).

In the present study, we investigated the effects of spinorphin and tynorphin on synaptic transmission in rat isolated hippocampal slices.

2. Materials and methods

2.1. Preparation of hippocampal slices

All experimental protocols were approved by the Animal Care and Use Committee of the Science University of Tokyo and were in accordance with the guidelines of the National Institutes of Health and of the Japanese Pharmacological Society.

Male Wistar rats (5 weeks) were anesthetized with urethane and α-chloralose and decapitated. The brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid of the following composition (mM): NaCl 124, KCl 5, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, glucose 10. The hippocampus was cut into slices 400 μm thick using a microslicer. The slices were kept in an incubation chamber containing artificial cerebrospinal fluid saturated with 95% O_2 and 5% CO_2 at 30°C for at least 1 h before use. Individual slices were then transferred to a recording chamber and perfused with artificial cerebrospinal fluid gassed with an O_2/CO_2 mixture at 30°C to yield a pH of 7.4. The slices were completely submerged in the medium and perfused continuously at a rate of 2.5 ml/min.

2.2. Stimulation and recording

Only slices exhibiting maximum action potentials above 3 mV at a stimulus strength of about 50 μA were used. Field potentials were recorded from the CA1 region following stimulation (0.1 ms) at 4–15-s intervals of Schaffer collaterals, using a glass microelectrode (1–5 MΩ) containing 2 M NaCl (Fig. 2). Stimulus strength was reduced to a level that yielded a spike size approximately 50% of the maximum (10–35 μA). To induce long-term potentiation, tetanic stimulation (100 Hz, 1 s) was used.

2.3. Data processing

The population spike amplitudes were measured using the Measurement and Analysis System for Extracellular Potentials (Furusawa Lab Appliance, Japan). The changes in population spike amplitude were expressed as percentages relative to that just before drug application or tetanic stimulation as 100%. The statistical significance of differences was determined by two-tailed Student's or Welch's *t*-test, or two-tailed Bonferroni's multiple *t*-test (Wallenstein et al., 1980).

2.4. Drugs

Spinorphin and tynorphin were supplied by Dr. Hazato (Tokyo Metropolitan Institute of Medical Science). [Leu^5]Enkephalin was obtained from Funakoshi. Naloxone hydrochloride was obtained from Research Biochemicals International. Amastatin, phosphoramidon and captopril were obtained from Sigma. All drugs were dissolved in artificial cerebrospinal fluid and perfused for 15 min. The application of the pretreatment drug was begun 5 min before of the start of enkephalin administration.

3. Results

3.1. Effects of spinorphin and tynorphin

Enkephalin (1–10 μM) increased the population spike amplitude dose dependently, and the effect was antago-

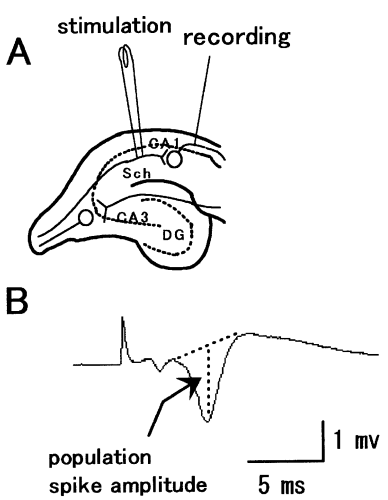

Fig. 2. Scheme showing positions of recording and stimulating electrodes in the hippocampus (A), and a sample trace of the population spike (B).

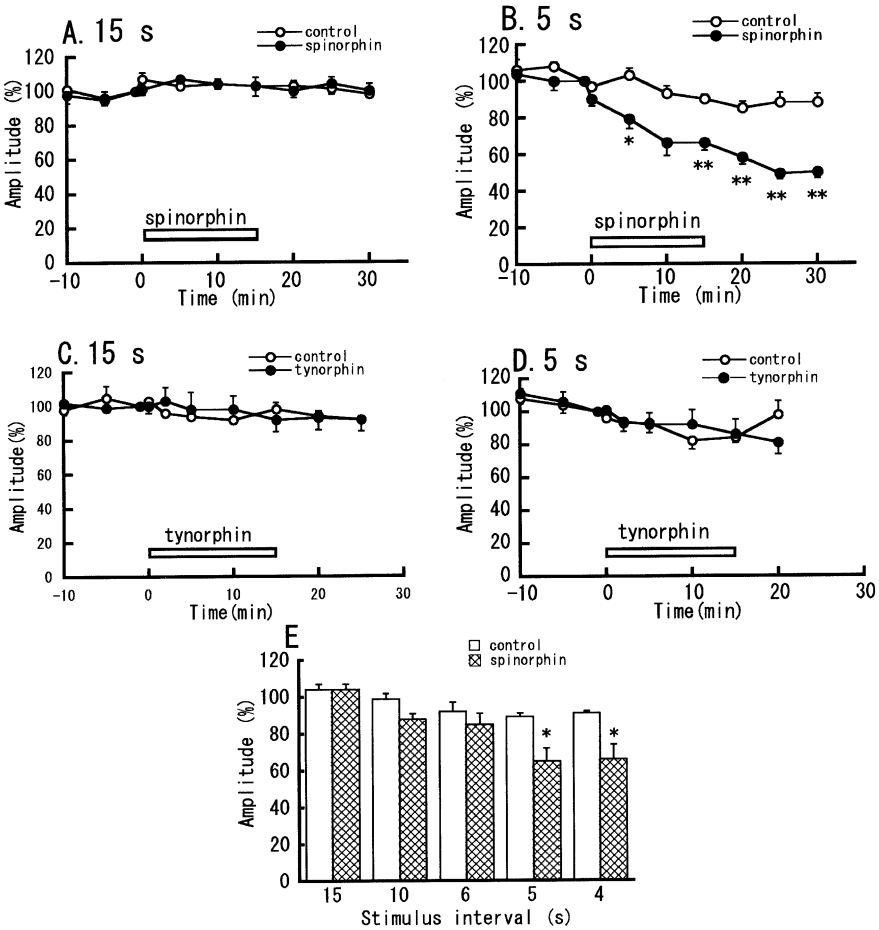

Fig. 3. Effects of spinorphin (1 μM) and tynorphin (1 μM) on population spike amplitude when the stimulus intervals were changed. Each point represents the mean ± S.E.M. for four rats in each group. The stimulus interval was 15 s (A, C) and 5 s (B, D), respectively. Drugs were perfused for 15 min as indicated (horizontal bar). (E) Percentage change in the population spike amplitude at 10 min. The statistical significance of differences was determined by two-tailed Student's or Welch's *t*-test. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control.

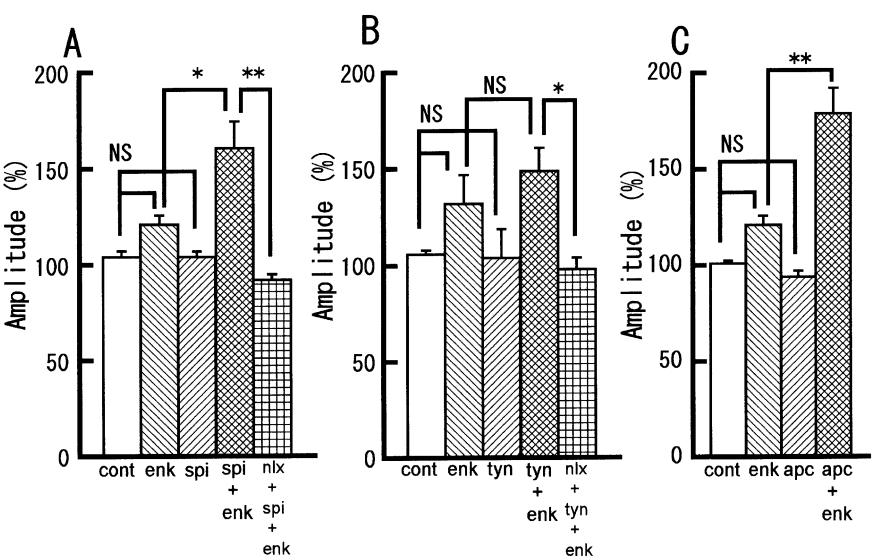

Fig. 4. Effects of spinorphin (1 μM, A), tynorphin (1 μM, B), and a mixture of amastatin (1 μM), phosphoramidon (1 μM) and captopril (1 μM) (C) on the facilitatory effect of enkephalin (1 μM) and blockade by naloxone (1 μM) at a stimulation interval of 15 s. Ordinates: population spike amplitude 10 min after enkephalin application. Each bar represents the mean ± S.E.M. for four rats in each group. cont: control, enk: enkephalin, spi: spinorphin, tyn: tynorphin, apc: a mixture of amastatin, phosphoramidon and captopril, nlx: naloxone. The statistical significance of differences was determined by two-tailed Bonferroni's multiple *t*-test (A and B: four comparisons in five groups, C: three comparisons in four groups). $^{*}P < 0.05$ and $^{**}P < 0.01$.

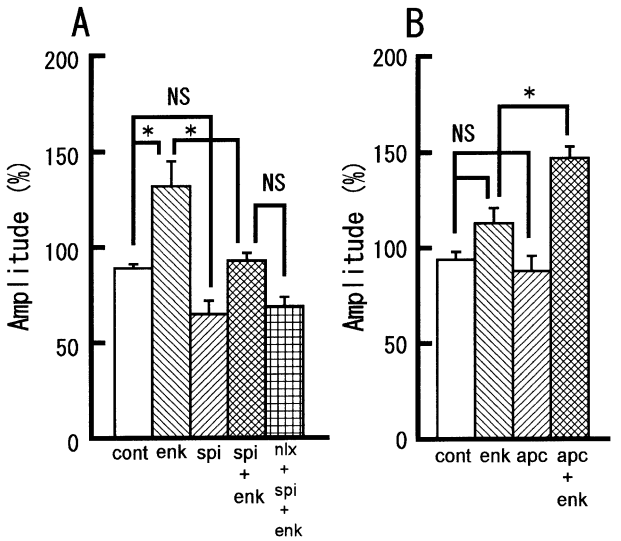

Fig. 5. Effects of spinorphin (1 μM, A) and a mixture of amastatin (1 μM), phosphoramidon (1 μM) and captopril (1 μM) (B) on the facilitatory effect of enkephalin at a stimulation interval of 5 s. Ordinates: population spike amplitude 10 min after enkephalin application. Each bar represents the mean ± S.E.M. for four rats in each group. cont: control, enk: enkephalin, spi: spinorphin, apc: a mixture of amastatin, phosphoramidon and captopril, n: naloxone. The statistical significance of differences was determined by two-tailed Bonferroni's multiple *t*-test (A: four comparisons in five groups, B: three comparisons in four groups). $^{*}P < 0.05$.

nized by the opioid receptor antagonist, naloxone (data not shown). Spinorphin (1 μM) had no effect on field potentials induced by stimulation at an interval of 15 s (Fig. 3A), but when the stimulation interval was shortened to 10, 6, 5 and 4 s, spinorphin reduced the amplitude of the field potentials frequency dependently (Fig. 3E). The inhibitory effects of spinorphin were not abolished by washout with artificial cerebrospinal fluid. At a 5-s stimulation interval, the inhibitory effect of spinorphin was dose-dependent and reached its maximum at 1 μM (data not shown). Tynorphin did not affect the field potential at any of the stimulus intervals employed (Fig. 3C,D).

3.2. Facilitatory effects of spinorphin and tynorphin on enkephalin

When the inhibitory effect of spinorphin did not appear at a stimulation interval of 15 s, spinorphin significantly potentiated the facilitatory effect of enkephalin and this effect was antagonized by naloxone (Fig. 4A). Tynorphin tended to potentiate the facilitatory effects of enkephalin, and these effects were antagonized by naloxone (Fig. 4A,B). On the other hand, a mixture of amastatin, phosphoramidon and captopril, synthetic inhibitors of enkephalin-degrading enzymes (Hiranuma and Oka 1986; Hiranuma et al., 1997, 1998), enhanced the effect of enkephalin (Fig. 4C).

3.3. Effects of spinorphin at high stimulation frequency

At a stimulation interval of 5 s, co-application of spinorphin and enkephalin had an additive effect on the inhibitory action of spinorphin and the facilitatory action of enkephalin. Naloxone antagonized only the effect of enkephalin and did not alter the inhibitory effect of spinorphin (Fig. 5A). On the other hand, a mixture of amastatin, phosphoramidon and captopril enhanced the effect of enkephalin (Fig. 5B).

3.4. Effect of spinorphin or tynorphin on induction of long-term potentiation

Stimulation intervals of 15 s were used in this experiment. Spinorphin (10 μM) and naloxone significantly

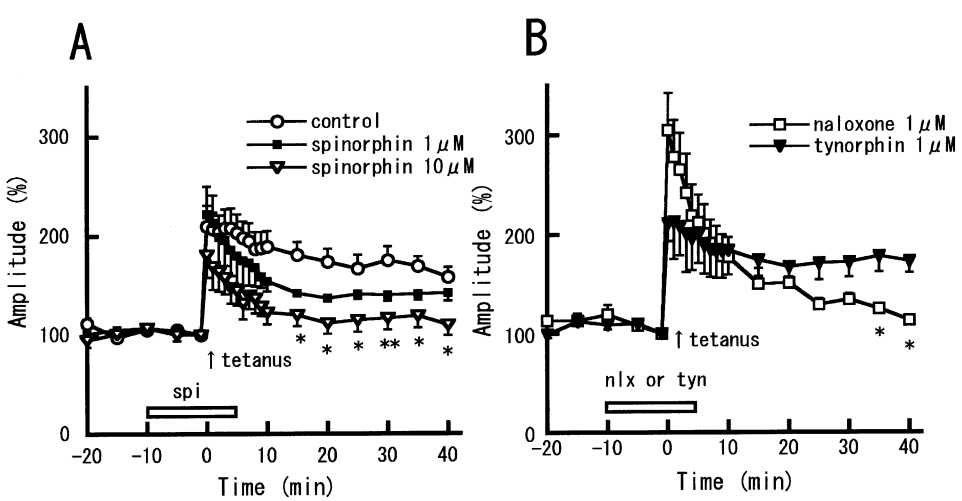

Fig. 6. Effects of spinorphin (1 and 10 μM, A), naloxone (1 μM, B) and tynorphin (1 μM, B) on the induction of long-term potentiation. Control was shown in A. Each point represents the mean ± S.E.M. for four (control: 8) rats in each group. Stimulus interval: 15 s, tetanus: 100 Hz 1s. Drugs were perfused for 15 min as indicated (horizontal bar). The statistical significance of differences between the control and drug-treated groups was determined by two-tailed Bonferroni's multiple *t*-test (four comparisons in five groups). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control.

reduced the long-term potentiation induced by tetanic stimulation (Fig. 6A,B). Spinorphin (1 μM) tended to reduce long-term potentiation (Fig. 6A), but tynorphin had no effect (Fig. 6B).

4. Discussion

Spinorphin has been isolated from the bovine spinal cord as an endogenous inhibitor of enkephalin-degrading enzymes. The K_i values of spinorphin on the aminopeptidase, dipeptidyl aminopeptidase III, angiotensin-converting enzyme and enkepalinase were 1.2, 0.51, 0.87 and 3.6 μM, respectively, on the enzymes purified from monkey brain (Nishimura and Hazato, 1993). Tynorphin selectively inhibited dipeptidyl aminopeptidase III at K_i value of 0.075 μM, and did not inhibit other enzymes at the concentration of 10 μM (Yamamoto et al., 2000). In the present study, 1–10 μM of spinorphin and 1 μM of tynorphin were employed.

In this study, enkephalin increased the synaptic responses and its effect was blocked by the opioid receptor antagonist, naloxone. At a stimulation interval of 15 s, spinorphin (1 μM), which alone had no effect, potentiated the facilitatory effect of enkephalin on the field potential (Fig. 4A). The effect of enkephalin + spinorphin was also antagonized by naloxone, suggesting that spinorphin potentiated the effect of enkephalin by preventing its degradation. Tynorphin (1 μM) tended to potentiate the facilitatory effect of enkephalin, which was antagonized by naloxone (Fig. 4B). These results suggest that tynorphin also potentiates the facilitatory effect of enkephalin by preventing its degradation. It is suggested that the facilitatory effect of tynorphin was smaller than that of spinorphin, since tynorphin blocks dipeptidyl aminopeptidase III and spinorphin blocks all enkephalin-degrading enzymes (aminopeptidase, dipeptidyl aminopeptidase III, angiotensin-converting enzyme and enkephalinase). This result was consistent with reports indicating that one inhibitor of enkephalin-degrading enzyme was unable to prevent the degradation of enkephalin (Hiranuma and Oka, 1986; Hiranuma et al., 1997, 1998).

At a stimulus interval of 5 s, spinorphin use dependently reduced the field potential (Fig. 3A,B,E). Co-application of spinorphin and enkephalin had an additive effect on the inhibitory action of spinorphin and the facilitatory action of enkephalin (Fig. 5A). Application of naloxone reduced the population spike amplitude to the size induced by spinorphin alone, suggesting that naloxone antagonized only the effect of enkephalin but had no action on the inhibitory effect of spinorphin. Tynorphin showed no use-dependent depression of the field potential (Fig. 3C,D). A mixture of amastatin, phosphoramidon and captopril is able to totally prevent the degradation of enkephalin (Hiranuma and Oka, 1986; Hiranuma et al., 1997, 1998), and spinorphin inhibits all the enkephalin-degrading enzymes (Nishimura and Hazato 1993). However, a mixture of amastatin, phosphoramidon and captopril potentiated the facilitatory effect of enkephalin at a stimulus interval of either 15 or 5 s (Figs. 4C and 5B), and showed no use-dependent inhibitory effects. Thus, the use-dependent inhibitory effect of spinorphin was considered to be peculiar to this agent, and to be unrelated to enkephalin-degrading enzymes. There are many drugs which have use-dependent effects, for example a Ca^{2+} channel blocker (Ishibashi et al., 1998), *N*-methyl-D-aspartate (NMDA) receptor antagonists (MacDonald and Nowak, 1990) and γ-aminobutyric acid-related drugs (MacIver et al., 1996), and it has been reported that influx or outflow of ions via open or activated channels is related to these effects. Therefore, further studies are needed to clarify the use-dependent inhibitory mechanism of spinorphin.

We also studied the effects of spinorphin and tynorphin on long-term potentiation, which is a form of synaptic plasticity following repetitive, high-frequency stimulation of excitatory presynaptic fibers. It produces long-lasting enhancement of synaptic transmission that is usually revealed as an increase in the size of excitatory post-synaptic potentials (Bliss and Lømo, 1973; Esposito and Pulvirenti, 1992). There are two types of long-term potentiation in the hippocampus: NMDA-dependent and NMDA-independent long-term potentiation (Martinez and Derrick, 1996). In the opioid-containing fiber input, naloxone blocks long-term potentiation (Xie and Lewis, 1995), since enkephalins released during high-frequency stimulation are one factor involved in the induction of long-term potentiation (Derrick and Martinez, 1994a,b). Since the opioid-containing fibers project to the CA1 region (Bramham, 1992; Francesconi et al., 1997), we expected that long-term potentiation would be potentiated by spinorphin and tynorphin, which would have prevented the degradation of enkephalins released during tetanus. However, we found that 1 μM of spinorphin tended to block long-term potentiation and 10 μM of spinorphin significantly blocked it, whereas tynorphin (1 μM) had no effect (Fig. 6A,B). The results are inconsistent with the idea that spinorphin and tynorphin inhibit the degradation of enkephalin and consequently potentiate long-term potentiation. Naloxone, which antagonizes endogenous enkephalins released during tetanus, significantly blocked long-term potentiation (Fig. 6B). The inhibitory effect of spinorphin but not tynorphin is considered to be due to its use-dependent inhibitory effect during tetanic stimulation, since the early phase of potentiation (post-tetanic potentiation) was also reduced by 10 μM of spinorphin.

The present results suggest that, at a low stimulation frequency, spinorphin potentiates the facilitatory effects of enkephalin on synaptic transmission by preventing the degradation of enkephalin, whereas, at a high stimulation frequency, spinorphin exerts use-dependent inhibitory effects that are unrelated to opioids. Unlike spinorphin, tynorphin has slight facilitatory effects on enkephalin and does not produce use-dependent inhibition.

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