Communication

Effects of $\beta$-Casomorphin-5 on Passive Avoidance Response in Mice

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The effects of intracerebroventricular (i.c.v.) injection of bovine $\beta$-casomorphin-5 ($\beta$-CM-5; Tyr-Pro-Phe-Pro-Gly), a $\mu$-opioid agonist derived from milk $\beta$-casein, on step-down type passive avoidance tasks were investigated in mice. Intracerebroventricular administration of a high dose (10 $\mu$g) of $\beta$-CM-5 produced a significant decrease in step-down latency. $\beta$-Funaltrexamine (5 $\mu$g, i.c.v.) almost completely reversed the $\beta$-CM-5-induced shortening of step-down latency, although neither naltrindole (5 ng, i.c.v.) nor nor-binaltorphimine (5 $\mu$g, i.c.v.) had any significant influence on the effect of $\beta$-CM-5. Meanwhile, a low dose (0.5 $\mu$g, i.c.v.) of $\beta$-CM-5 inhibited scopolamine (1 mg/kg)-induced impairment of passive avoidance response. These results indicated that a high dose of $\beta$-CM-5 induces amnesia, whereas a low dose ameliorates scopolamine-induced amnesia.

Key words: $\beta$-casomorphin-5; $\mu$-opioid receptor; passive avoidance response; step-down latency; scopolamine

$\beta$-Casomorphins ($\beta$-CMs) belong to the family of opioid peptides derived from food protein. Bovine $\beta$-CM-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) was first isolated from an enzymatic digest of bovine $\beta$-casein. Shorter fragments, such as the N-terminal pentapeptide (bovine $\beta$-CM-5), were also found to be active, in fact more active than $\beta$-CM-7. These peptides have been found to have a $\mu$-opioid receptor agonist activity in isolated organs, and binding experiments. $\beta$-CM-5 is known to have an antinociceptive effect after intracerebroventricular administration.

It is now known that opioid neuronal systems play an important role in memory processes. $\mu$- and $\delta$-Opioid receptor agonists have been reported to impair learning and memory, whereas $\kappa$-opioid receptor agonists improve the impaired memory. Recently, oral administration of gluten exorphin A5, one of the opioid peptides derived from wheat gluten, has been reported to facilitate the acquisition/consolidation process of learning and memory. In this study, we report the effects of central injections of $\beta$-CM-5 on the passive avoidance response in mice.

Six-week-old male ddY mice (Nihon SLC, Japan) weighing about 28–33 g were kept in a regulated environment (24 ± 1°C, 55 ± 5% humidity), with a 12-h light/12-h dark cycle (lights on, 6:00 a.m.–6:00 p.m.) and given food and tap water ad libitum. The mice were kept at least 7 days in home cages before starting experiments. Experimental protocols concerning the use of laboratory animals were approved by the Experimental Animal Committee of the Osaka University of Pharmaceutical Sciences. $\beta$-CM-5 (Peptide Institute, Minoh, Japan), $\beta$-funaltrexamine ($\beta$-FNA), naltrindole, nor-binaltorphimine (nor-BNI) (Tocris Cookson Inc. Ballwin, MO, USA), and scopolamine (Wako Pure Chemicals, Osaka, Japan) were dissolved in saline. The intracerebroventricular (i.c.v.) injections were made according to the method of Maurice et al. Drug solutions (5 $\mu$l/mouse) were delivered gradually within approximately 3 s with a 50-$\mu$l microsyringe (705LT; Hamilton Co., Reno, NV, USA). Scopolamine was injected subcutaneously (s.c.) in a volume of 100 $\mu$l/30 g of body weight. The effects of $\beta$-CM-5 on learning and memory were investigated using step-down type passive avoidance tasks. The dark compartment of the step-through passive avoidance apparatus for rats (Cat. 7550, Ugo Basile, Italy) was substituted for the step-down passive avoidance apparatus. The apparatus consisted of a cage made of black Perspex panels (23 × 21 × 22 cm high) with a grid floor, inserted in a soundproof outer box (40 × 90 × 100 cm high). The cage was illuminated with a 30 W lamp during the experimental period. A wooden platform (4 × 4 × 4 cm) was fixed at the center of the grid floor. Electric shocks (0.3 mA) were delivered to the grid floor. The test consisted of a training session and a retention session, done 24 h after the training. During the training session, each mouse was placed on the platform. When it stepped down and placed its four

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Abbreviations: $\beta$-CM, $\beta$-casomorphin; i.c.v., intracerebroventricular or intracerebroventricularly; $\beta$-FNA, $\beta$-funaltrexamine; nor-BNI, nor-binaltorphimine
paws on the grid floor, an electric shock was delivered for 9 sec. The retention test was done in a similar manner, except that no electric shock was applied to the grid floor. Each mouse was placed again on the platform, and the step-down latency (SDL) was recorded, with an upper cut-off time of 300 sec. β-CM-5 and scopolamine were administered immediately after and 30 min before the training session, respectively. β-FNA, naltrindole and nor-BNI were injected 24 h, 20 min and 60 min before training, respectively, according to the method of Ukai et al. Fig. 1: Effects of β-CM-5 on Step-down Latency in a Passive Avoidance Task in Mice. β-CM-5 (i.c.v.) was given to mice immediately after training. Data are expressed as the median, first, and third quartiles. The number of mice used is shown in parentheses.* P < 0.05 vs. control (Scheffé's test).

Fig. 2: Effects of β-FNA (A), Naltrindole (B), or nor-BNI (C) on β-CM-5-Induced Impairment of Step-down Latency in a Passive Avoidance Task in Mice. β-FNA (i.c.v.), naltrindole (i.c.v.) and nor-BNI (i.c.v.) were injected 24 h, 20 min and 60 min before training, respectively, according to the method of Ukai et al.11) SDL was expressed as the median, first, and third quartiles. The significance of differences was evaluated using the two-tailed Mann-Whitney U-test for paired comparisons and the Kruskal-Wallis non-parametric one-way analysis of variance followed by Scheffé's test for multiple comparisons. The criterion for significance was P < 0.05 in all statistical evaluations.

The effects of intracerebroventricular administration of β-CM-5 on the passive avoidance response were investigated. β-CM-5 (10 μg) significantly shortened the SDL (Kruskal-Wallis analysis: H = 10.0, P < 0.05) (Fig. 1). To elucidate the mechanism of impairment by β-CM-5, the effects in combination with β-FNA, naltrindole, or nor-BNI (a μ-, δ-, or κ-opioid receptor antagonist, respectively) on the passive avoidance performance were investigated. β-FNA (5 μg, i.c.v.) almost completely reversed the effect of β-CM-5 (10 μg) (Kruskal-Wallis analysis: H = 12.5, P < 0.01) (Fig. 2A), although naltrindole (5 ng, i.c.v.) and nor-BNI (5 μg, i.c.v.) had no significant effect (Fig. 2B, C). β-FNA, naltrindole, or nor-BNI alone had no significant effect on the SDL (Fig. 2A, B, C). In order to test whether or not β-CM-5 ameliorates amnesia, we further investigated its effect on the scopolamine-induced impairment of the passive avoidance response. Scopolamine (1 mg/kg, s.c.) significantly shortened the SDL. β-CM-5 (0.1–10 μg/mouse) administered immediately after
The use of maze tasks using post-training administration, retention of memory was significant (Fig. 3).

Interestingly, these results also showed that a low dose of β-CM-5 (0.5 µg, i.c.v.), which alone did not influence the SDL (data not shown), alleviated the scopolamine-induced impairment of the passive avoidance response.

Cholinergic neurotransmission is thought to be important for learning and memory. Many investigators have studied the interaction between opioids and the cholinergic system in memory performance. Morphine has been shown to decrease the output of acetylcholine in areas of the brain connected to learning and memory. Muscarinic agonists antagonize µ-opioid receptor agonist-induced memory impairment. Therefore, intracerebroventricular injection of a high dose (10 µg) of β-CM-5 may result in cholinergic dysfunction mediated via µ-opioid receptors.

The ameliorative effect of the low dose of β-CM-5 on scopolamine-induced amnesia cannot be explained by the inhibitory action of the opioid on cholinergic neurotransmission. Biphasic dose-dependent inhibitory and stimulatory effects of opioids on electrophysiological responses of dorsal root ganglion neurons in culture have been described by Crain and Shen.

Low concentrations of opioid agonists prolong the calcium-dependent component of the action potential duration (APD) and stimulate transmitter release, whereas higher levels shorten the APD and inhibit transmitter release. Therefore, low levels of opioids may stimulate synaptic function concerning learning and memory. Furthermore, evidence from several studies suggest that opioids affect memory storage through interaction with other neurotransmitter systems, including adrenergic, GABAergic and dopaminergic systems. It is known that µ-opioid receptor agonists have an excitatory effect on pyramidal neurons in the hippocampus due to inhibition of the release of GABA from interneurons. There is a possibility that β-CM-5 inhibits the proteolytic inactivation of neuropeptides in the brain. Many neuropeptides are known to facilitate learning and memory. For example, arginine-vasopressin and substance P have been shown to affect these processes, and were suggested to be inactivated by prolyl endopeptidase (PEP). In fact, treatment with specific PEP inhibitors improved learning and memory. Interestingly, it was shown that peptides containing the amino acid sequence of β-CM-5 have inhibitory activity against PEP. Therefore, further study of the involvement of neurotransmission systems other than opioid-opioid receptor systems is required to clarify the mechanisms of the anti-amnesic effect of β-CM-5.

Fig. 3. Effects of β-CM-5 on Scopolamine-Induced Impairment of Step-down Latency in a Passive Avoidance Task in Mice. β-CM-5 (i.c.v.) and scopolamine (1 mg/kg, s.c.) were given to mice immediately after and 30 min before training, respectively. Data are expressed as the median, first, and third quartiles. The number of mice used is shown in parentheses. *P<0.05, **P<0.01 vs. control (Scheffe’s test), †P<0.05 vs. scopolamine alone (Mann-Whitney U-test).

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References


