\(\beta\)-Endorphin Involvement in the Antidopaminergic Effect of Caerulein

Katsuaki MATSUBARA and Akira MATSUSHITA
Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan
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Abstract—Caerulein has been shown to possess a long-lasting antagonistic effect on amphetamine hyperactivity in rats when given in combination with haloperidol. We found that this effect of caerulein involved \(\beta\)-endorphin. Naloxone pretreatment and hypophysectomy abolished the caerulein effect, while intracerebroventricular or intra-nucleus accumbens injection of \(\beta\)-endorphin together with haloperidol administration produced an effect similar to that of caerulein. The results suggest that the long-term antagonism of the amphetamine effect of caerulein is mediated by the endogenous opioid \(\beta\)-endorphin.

Caerulein (CLN), a decapeptide chemically related to cholecystokinin octapeptide (CCK-8), has been found to result in a long-lasting antagonistic effect on amphetamine (AMP) hyperactivity in rats when given together with haloperidol (HLP) (1). Also, we reported the pharmacological analysis of the nature of this long-term effect (2). In addition, a recent study showed that CLN may indirectly modulate some dopaminergic activity in the central nervous system (3). However, at present, the mechanism producing this effect remains to be elucidated.

CLN and CCK-8 have been shown to directly affect the anterior pituitary lobe or the hypothalamus in rats, suggesting that they play an important role in regulating the secretion of pituitary hormones (4–6). Furthermore, in humans, CLN significantly increases \(\beta\)-endorphin (\(\beta\)-END) levels in plasma and cerebrospinal fluid (7–9). It was also demonstrated that \(\beta\)-END modulates some dopaminergic systems to influence a variety of behavioral phenomena, i.e., changes in motoric behavior such as stereotypy, cataleptic posturing, and spontaneous activity (10, 11).

Thus, our interest was directed to search for interaction between \(\beta\)-END and the CLN effect. The present study was done to investigate whether \(\beta\)-END is involved in producing the long-term effect of CLN on AMP-induced hyperactivity in rats.

Materials and Methods

Animals: Male Wistar rats, purchased from Shizuoka Laboratory Animal Center, Japan, were 250–300 g at the beginning of the experiments. They were housed in groups with free access to food and water on an 8 a.m.–8 p.m. lighting schedule during the experiments.

Hypophysectomy: Hypophysectomy was performed through the external auditory canal by the modified method of Tanaka (12). The animals were allowed at least 1 week to recover from the operation before the experiment.

Cannulae implantation: A stainless steel guide cannula for the right lateral ventricle or bilateral cannulae for the nucleus accumbens was implanted using the optical brain tracer technique (13). The injection units, 0.31 mm in diameter, were terminated 1.5 mm below the guide tips, so that the drug was deposited at the site with the following König and Klippel coordinates (14): A 6.4, L 1.4, H 1.8, in the lateral ventricle; A 9.4, L 1.4, H −0.6, in the nucleus accumbens. The animals were allowed 1 week to recover before testing. \(\beta\)-END was injected into the lateral ventricle in a volume of 5 \(\mu\)l over a period of 30 sec or...
into the nucleus accumbens bilaterally in a volume of 0.4 µl over a period of 10 sec through an injection cannula passed into each guide cannula.

Experimental procedures: On the 1st day, intact animals in four equal groups (n=24) were administered with naloxone (NX, 0.5 mg/kg) and then 15 min later, were injected with saline, HLP (0.1 mg/kg), CLN (40 µg/kg), or a combination of CLN and HLP. After 60 min, all groups received AMP (2 mg/kg). At 30 min after AMP administration, each rat was placed in an open field apparatus (15), and its ambulatory activity was measured for 3 min. On the 2nd, 8th or 15th day, each rat was injected with AMP (2 mg/kg) alone, and 30 min later, the activity was measured as on the 1st day. Hypophysectomized rats in four equal groups (n=20) were subjected to experimental procedures similar to those for the intact rats except for pretreatment with NX. Six equal groups of rats (n=30) with intracerebroventricular or intra-nucleus accumbens injection units were given β-END instead of CLN into the brain, with the other procedures being the same as those for intact rats except for NX pretreatment.

Drugs: Caerulein (ceruletide diethylamine, synthesized in our laboratory), dl-amphetamine sulfate (Zedrin, Takeda), haloperidol (Shionogi), naloxone hydrochloride (Endo Laboratory), and β-endorphin (Osaka Protein Research Foundation) were used. All drugs were dissolved in saline and administered subcutaneously except for intracranial injection of β-END.

Statistical analysis: The experimental results were analyzed by Dunnett's t-test for multiple comparison following one-way analysis of variance.

Results

Effect of naloxone: Previous reports (1, 2) described the long-lasting antagonistic effect of CLN on AMP-induced hyperactivity in rats, when the peptide was given in combination with HLP, and briefly mentioned the dose-dependency of the duration of CLN action; the effect lasted for 14 days at 40 µg/kg of CLN.

In the present experiment with intact animals pretreated with NX (0.5 mg/kg), drug treatment with HLP or a combination of HLP and CLN significantly reduced AMP-induced hyperactivity on the first day in comparison with the saline-treated control group (Fig. 1; F=48.21, df 3/20, P<0.01). On the 2nd day, however, no significant reduction in the AMP effect was found in the HLP plus CLN group (Fig. 1; F=0.76, df 3/20, P>0.05), unlike the results of the previous reports (1, 2). Systemic administration of the opiate antagonist NX blocked the long-term antagonistic effect of CLN on AMP-induced hyperactivity.

Effect of hypophysectomy: On the 1st day, the hypophysectomized rats treated with HLP or HLP plus CLN were less sensitive to AMP (Fig. 2; F=18.92, df 3/20, P<0.01). On the 2nd day, no significant differences were found among the four groups (Fig. 2; F=0.89, df 3/20, P>0.05). Hypophysectomy abolished the long-term effect of CLN on AMP-induced hyperactivity, similar to the result of NX pretreatment.

Effect of intracerebroventricular or intra-

![Graph](https://example.com/graph.png)
nucleus accumbens injection of β-endorphin:
Animals intracerebroventricularly injected with 0.625, 2 or 10 μg of β-END on the 1st day showed normal responses to AMP, but those given HLP alone or HLP plus β-END showed significantly reduced responses (Fig. 3; F=20.63, df 7/32, P<0.01). On the 2nd day, four groups given HLP alone or each dose of β-END alone on the 1st day displayed normal susceptibility to AMP, whereas the groups treated with HLP plus each dose of β-END were still less susceptible to AMP (Fig. 3; F=19.81, df 7/32, P<0.01). On the 8th day, the response to AMP of animals that received HLP plus 10 μg of β-END was still significantly reduced (Fig. 3; F=2.41, df 7/32, P<0.05), but normal susceptibility was regained by the 15th day (F=0.37; df 7/32, P>0.05). Thus, the duration of long-lasting reduction in AMP hyperactivity elicited by both injection of HLP and intracerebroventricular injection of β-END depended on the β-END dose on the 1st day.

In a separate experiment, animals were
given intra-nucleus accumbens injections of 0.02, 0.2 or 2 μg of β-END on the 1st day. The anti-AMP activity of β-END alone was observed only in the 1st day test of the groups given 0.2 and 2 μg. This reduction in AMP hyperactivity is due to an acute β-END effect and differs in nature from the effect investigated here. In addition, the response to AMP of animals given HLP alone or HLP plus each dose of β-END was significantly reduced (Fig. 4; F=20.63, df 7/32, P<0.01). On the 2nd day, four groups given HLP alone or each dose of β-END alone on the 1st day showed normal susceptibility to AMP, whereas the groups treated with HLP plus each dose of β-END were still less susceptible (Fig. 4; F=26.25, df 7/32, P<0.01). On the 8th day, the response to AMP of animals given HLP plus 0.2 or 2 μg of β-END was still significantly reduced (Fig. 4; F=29.64, df 7/32, P<0.01), but normal response to AMP was regained by the 15th day. Thus, these results, like those for animals given intracerebroventricular injection of β-END, clearly demonstrated that the duration of long-term reduction in AMP hyperactivity induced by intra-nucleus accumbens-injection of β-END together with HLP depended on the 1st day.

Discussion

Subcutaneous administration of either CLN or CCK-8 has been shown to produce NX-sensitive antinociceptive effects in mice (16, 17). CCK-8-induced effects on both food intake and forestomach motility in sheep was abolished after NX pretreatment (18). These observations suggest that part of the pharmacological effects induced by CLN or CCK-8 is mediated by an opiate receptor or a release of endogenous opioid peptides. In humans, the analgesic action of CLN was found to be completely antagonized by NX pretreatment, and CLN administration significantly augmented β-END levels (9).

The present findings indicate that NX pretreatment before the experimental paradigm on the 1st day abolishes the long-term effect of CLN on AMP-induced hyperactivity, suggesting that the CLN effect in this experiment may be also involved in endogenous opioids.

β-END occurs in the highest concentrations in the anterior and neurointermediate
lobe of the pituitary. Moreover, its pituitary concentrations are two magnitudes higher than those of other peptides in the pituitary or central nervous system (19, 20). In the neurointermediate pituitary, β-END can be α-N-acetylated further at its N-terminus. This modification causes it to lose all its opiate-like properties (21–23).

CCK-8 has been shown to increase plasma levels of ACTH-like activity, when given intraperitoneally (4). The effect is probably caused by direct action on the anterior pituitary lobe or the hypothalamus. Moreover, CCK-8 causes release of β-END immunoreactivity from the anterior pituitary (6).

In the second set of experiments, we used hypophysectomized animals to determine whether pituitary is indispensable for producing the long-lasting effect of CLN on AMP-induced hyperactivity. As CLN in combination with HLP could not induce the long-term effect in these animals, the release of β-END from the anterior pituitary may play an important role in the production of the CLN effect.

Since CLN plays a role in regulating β-END secretion, the third set of experiments were done to examine whether intracranial-injection of β-END mimics the action of CLN in the present experimental paradigm. Like the effect of CLN (1, 2), both intraventricular and intra-nucleus accumbens injection of β-END produced the long-term and dose-dependent reduction of AMP-induced hyperactivity with a smaller dose being needed for the intra-nucleus accumbens injection.

These results suggest that at least the release of β-END in the nucleus accumbens may be involved in the CLN effect since enhanced locomotor activity elicited by AMP results from releasing dopamine from dopamine nerve terminals in the nucleus accumbens (24–26) and in addition, opiate receptors are located presynaptically on dopaminergic neurons from the nucleus accumbens with mediating presynaptic inhibition of dopamine release (27). At present, no evidence is available for the β-ENDergic relationship between the nucleus accumbens and the anterior pituitary, and the reason why the long-lasting effect of CLN occurs is still not known.

In conclusion, our study has demonstrated that NX pretreatment and hypophysectomy abolish the CLN effect, and β-END mimics the long-term effect of CLN, suggesting that the CLN effect may result from mediation by the endogenous opioid β-END.

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