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# Ceruletide Inhibits Phencyclidine-Induced Dopamine and Serotonin Release in Rat Prefrontal Cortex

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ETOU, K., T. KUROKI, T. KAWAHARA, Y. YONEZAWA, N. TASHIRO AND H. UCHIMURA. *Ceruletide inhibits phencyclidine-induced dopamine and serotonin release in rat prefrontal cortex.* PHARMACOL BIOCHEM BEHAV **61**(4) 427–434, 1998.—Phencyclidine (PCP; 5.0 mg/kg, IP) produced a greater increase in extracellular dopamine (DA) levels in the prefrontal cortex than in the striatum, while PCP increased the extracellular 5-hydroxytryptamine (serotonin; 5-HT) levels in the prefrontal cortex but not the striatum, as determined by in vivo microdialysis in awake, freely moving rats. The cholecystokinin (CCK)-related decapeptide ceruletide (120 and 400 μg/kg, IP), administered 60 min prior to PCP, significantly attenuated the PCP-induced increase in the extracellular levels of DA and 5-HT in the prefrontal cortex, but not in the striatum. These effects were reversed by PD 135,158, a selective CCK-B receptor antagonist (0.1 mg/kg, SC), administered 5 min prior to ceruletide. When administered alone, ceruletide (400 μg/kg, IP) significantly increased basal extracellular DA levels only in the prefrontal cortex. The selective *N*-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (0.5 mg/kg, IP) also increased extracellular DA levels in the prefrontal cortex, but this effect was unaffected by ceruletide pretreatment. These results suggest that ceruletide may differentially modulate basal and PCP-induced release of DA and 5-HT in the prefrontal cortex. © 1998 Elsevier Science Inc.

Cholecystokinin Ceruletide Phencyclidine Dizocilpine Dopamine Serotonin Release Prefrontal cortex Striatum In vivo microdialysis Rat

CHOLECYSTOKININ (CCK) may influence the dopaminergic activity because CCK has been demonstrated to coexist with dopamine (DA) in a large proportion of neurons in the ventral tegmental area and substantia nigra in both rodent and primate brain (5,14). CCK octapeptide sulfate form (CCK-8-S) and its related decapeptide ceruletide have been reported to decrease DA release in the striatum (2,11) and increase DA release in the prefrontal cortex (10) as well as in the nucleus accumbens (12,25). CCK-8-S and ceruletide have also been reported to inhibit haloperidol or amphetamine-induced DA release in the striatum and the nucleus accumbens (2,21,22). These data may thus imply a modulatory effect of CCK on the dopaminergic activity in a region-specific manner. Therefore, CCK and its related peptide have been suggested to be involved in the pathophysiology of various neuropsychiatric disorders (8,42) and to also have an antipsychotic potential (28).

1-(1-Phenylcyclohexyl)piperidine hydrochloride (phencyclidine; PCP) has been one of the major drugs involved in substance abuse in the USA since the late 1960s. This drug has received attention because it can produce psychosis that closely resembles both the positive and negative symptoms of schizophrenia (17). Therefore, PCP has been suggested to be useful as an animal model of schizophrenia (40). PCP, like amphet-

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amines, causes behavioral effects such as hyperlocomotive and stereotyped behaviors in rodents. It should be noted that both systemic and local administration of PCP has been reported to produce greater increases in the release and metabolism of DA in the prefrontal cortex than in the striatum (7,15,30). Prefrontal cortex DA neurons have been suggested to play an essential role in the cognitive functions (6,29). Therefore, the preferential activation by PCP in the prefrontal cortex DA neurons may be related to the basis for the symptomatology of PCP-induced psychosis, although the precise mechanism for this remains unclear.

Previous studies have demonstrated that CCK could modulate the behavioral and neurochemical effects of PCP. CCK, bilaterally injected into the nucleus accumbens, significantly inhibited both PCP-induced circling and ataxia in rats, most likely due to the stimulation of the CCK-B receptors (34). Incubation with PCP attenuated $K^+$-induced CCK release from slices of rat cerebral cortex (1), and systemic administration of PCP decreased CCK-like immunoreactivity in the frontal cortex and the nucleus accumbens (45). Moreover, ceruletide attenuated PCP-induced increases in DA metabolism in the nucleus accumbens and the prefrontal cortex (23). Based on the above-mentioned interactions between CCK and PCP, it could be hypothesized that CCK specifically modulates PCP-induced DA release in the mesolimbocortical DA neuron systems. If so, the CCK-related peptide such as ceruletide would be useful for the treatment of PCP-induced psychosis and other psychiatric disorders.

CCK may also interact with the serotonergic systems because CCK-A receptors are located on 5-hydroxytryptamine (serotonin; 5-HT) neurons in the dorsal raphe nucleus of the rat brain (3), and the stimulation and blockade of CCK-B receptors have been shown to modulate cortical 5-HT release in animal models of anxiety (36). CCK release may be mediated by $5\text{-HT}_3$ receptors in the rat cerebral cortex and nucleus accumbens (31). Because PCP has been reported to increase 5-HT release in the rat striatum and nucleus accumbens (13), it is possible that ceruletide also modulates PCP-induced 5-HT release in the specific brain regions.

This study was designed to examine whether and how ceruletide affects the PCP-induced changes in the extracellular levels of DA and 5-HT in the prefrontal cortex and striatum of awake, freely moving rats, using in vivo brain microdialysis. We also examined the effect of ceruletide on the selective non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(a,d)cycloheptan-5,10-imine hydrogen maleate (dizocilpine; MK-801)-induced changes in extracellular levels of DA and 5-HT in the prefrontal cortex, because the noncompetitive antagonism by PCP of NMDA receptors has been suggested to be involved in the mechanism(s) of PCP-induced prefrontal cortex DA release (16,48).

## METHOD

### *Animals and Surgery*

Male Wistar rats weighing 250–350 g were housed three or four per cage at constant room temperature (22°C) and relative humidity (50%) under a 12h light-dark cycle (lights: 8:00–20:00h), and were provided with food and water ad lib. The rats were anesthetized intraperitoneally with chloral hydrate (300 mg/kg) and placed on a stereotaxic frame. A concentric dialysis probe (regenerated cellulose membrane, 3.0 mm in length, 0.22 mm in outer diameter, cutoff molecular weight 50,000; Eicom Co., Kyoto, Japan) was then implanted into either the left medial prefrontal cortex (coordinates: A +2.7 mm, L +0.7 mm from bregma, 4.2 mm below dura surface) or the left striatum (coordinates: A +1.0 mm, L +2.5 mm from bregma, 5.0 mm below dura surface) of the rat brain, according to the reference atlas (32). The rats were monitored until full recovery from the anesthesia for at least 20-h, after which the extracellular DA levels in the dialysates were observed to be stabilized (no more than 10% variation).

### *Microdialysis*

One day following the surgery, the implanted probe was perfused with an artificial cerebrospinal fluid (140 mM NaCl, 3.35 mM KCl, 1.26 mM $CaCl_2$, 1.15 mM $MgCl_2$, 1.2 mM $NaHPO_4$ and 0.3 mM $NaH_2PO_4$, at pH 7.3) at a flow rate of 1.5 μl/min in the prefrontal cortex and of 2.0 μl/min in the striatum, respectively. After a 2-h period of equilibration, dialysis samples were collected every 30 min from the prefrontal cortex and every 20 min from the striatum. After three consecutive samples were collected to determine the basal extracellular levels of DA and 5-HT, the drugs were then administered. Because the variability in % recovery of DA and 5-HT between probes varied by less than 10%, basal extracellular levels of DA and 5-HT in each region were expressed as absolute values and were not corrected by % recovery. After the completion of each experiment, the rats were sacrificed with an overdose of chloral hydrate. The brains were removed and cut coronally along the puncture created by the probe using a cryostat. The location of the tip was then verified macroscopically. All experiments were carried out between 9:00 and 17:00h. The procedures performed in this study were approved by the Comittee of Ethics on Animal Experiments of the Faculty of Medicine, Kyushu University and were in strict accordance with the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University and The Law (No. 105) and Notification (No. 6) of the Japanese Government.

### *Assay of Extracellular Levels of DA and 5-HT in Dialysates*

Dialysate samples were directly applied onto high-performance liquid chromatography with electrochemical detection (HPLC-ECD) using an on-line injector (CMA 160, BAS, Lafayette, IN), as previously described (48). DA and 5-HT were separated on a reverse phase column (CA-5 ODS, 1μm particle size, 4.6 × 150 mm, Eicom Co., Kyoto, Japan). The mobile phase consisted of 0.1 M phosphated buffer (pH 6.0), 20% (v/v) methanol, 500 mg/l sodium 1-octanesulfonate (S.O.S.), and 5 mg/l $Na_2EDTA$. Both monoamines were detected by ECD (LC-4B, BAS) with a graphite carbon electrode set at +500 mV vs. an Ag/AgCl reference electrode (CB-100, Eicom).

### *Drug Treatment*

Ceruletide diethylamine was generously denoted by Shionogi Research Laboratories (Osaka, Japan). 1-(1-Phenylcyclohexyl)piperidine hydrochloride (phencyclidine; PCP) was synthesized according to the method of Maddox et al. (24) and identified by nuclear magnetic resonance imaging and mass spectroscopy. (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(a, d) cyclo-heptan-5,10-imine hydrogen maleate (dizocilpine; MK-801), D,L-4-(3,4-dichlorobenzoylamino)-5-(diphentylamino)-5-oxo-pentanoic acid sodium salt (lorglumide), a selective CCK-A receptor antagonist, and 4-{[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[[1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]carbonyl] amino]propyl]amino]-1-phenylethyl]amino-4-oxo-[1S-1α,2β[*S**(*S**)]4α]}-butanoate *N*-methly-D-glucamine salt (PD

135,158), a selective CCK-B receptor antagonist, were purchased from Research Biochemicals Inc. (Natick, MA). All drugs were dissolved in saline and then intraperitoneally (IP) or subcutaneously (SC) administered to rats. The rats were IP pretreated with ceruletide 120, 400 μg/kg, or saline 60 min prior to the IP administration of PCP 5.0 mg/kg or MK-801 0.5 mg/kg. Lorglumide 1.0 mg/kg or PD 135,158 0.1 mg/kg was SC administered 5 min prior to ceruletide. A control group of rats received saline only according to the same regime. The doses of ceruletide were chosen based on the findings of the previous study, which showed them to reduce PCP-induced increases in tissue DA metabolism in rat prefrontal cortex (23). The selective CCK receptor antagonists at the doses chosen in this study were reported to produce the behavioral and neurochemical effects mediated by the antagonism of the specific receptor subtypes (16, 43). The dose of MK-801 would be equivalent to that of PCP for the antagonism of NMDA receptors according to the comparative studies on the behavioral effects of these compounds following systemic administration (41).

*Statistical Analysis*

All data are presented as percentages of the predrug basal levels (100%), calculated as the mean of the three consecutive samples before drug administration. The data were analyzed statistically using the two-way repeated measures analysis of variance (ANOVA) followed by Scheffe *F*-test. Treatment or dose was treated as a between-subject variable, while time was treated as a repeated-measures variable. A probability ($p$) of less than 0.05 was considered to be significant in the present study.

## RESULTS

*Basal Extracellular Levels of DA and 5-HT in the Prefrontal Cortex and the Striatum*

The predrug basal levels (mean ± SEM, not corrected for in vitro recovery by probe membrane) of extracellular DA and 5-HT were as follows: 2.18 ± 0.25 and 2.34 ± 0.10 pg/45 μl/30 min in the prefrontal cortex ($n$ = 47); 11.92 ± 0.91 and 2.10 ± 0.09 pg/40 μl/20 min in the striatum ($n$ = 32), respectively. Characterization of basal extracellular monoamine levels has been reported in elsewhere (48).

*Effects of PCP and MK-801 on Basal Extracellular Levels of DA and 5-HT in the Prefrontal Cortex and the Striatum (Figs. 1, 2, and 4).*

PCP (5 mg/kg, IP) remarkably increased basal extracellular DA levels in the prefrontal cortex [maximum increase during a 180-min period relative to the predrug basal levels: 1092 ± 200%; $F(1, 8) = 118.24$, $p < 0.001$]. PCP also produced a significant increase in basal extracellular DA levels in the striatum, compared to the saline control [219 ± 21%; $F(1, 8) = 64.70$, $p < 0.001$]. Thus, PCP increased extracellular DA levels in the prefrontal cortex to a greater extent than in the striatum. PCP (5 mg/kg, IP) caused a significant increase in basal extracellular 5-HT levels in the prefrontal cortex [165 ± 21%; $F(1, 7) = 13.85$, $p = 0.007$] but had no significant effect in the striatum, compared to the saline control.

MK-801 (0.5 mg/kg, IP) produced a significant increase in basal extracellular DA levels [265 ± 40%; $F(1, 8) = 17.41$, $p = 0.003$] in the prefrontal cortex, compared to the saline control. However, MK-801 did not affect basal extracellular 5-HT levels in the prefrontal cortex.

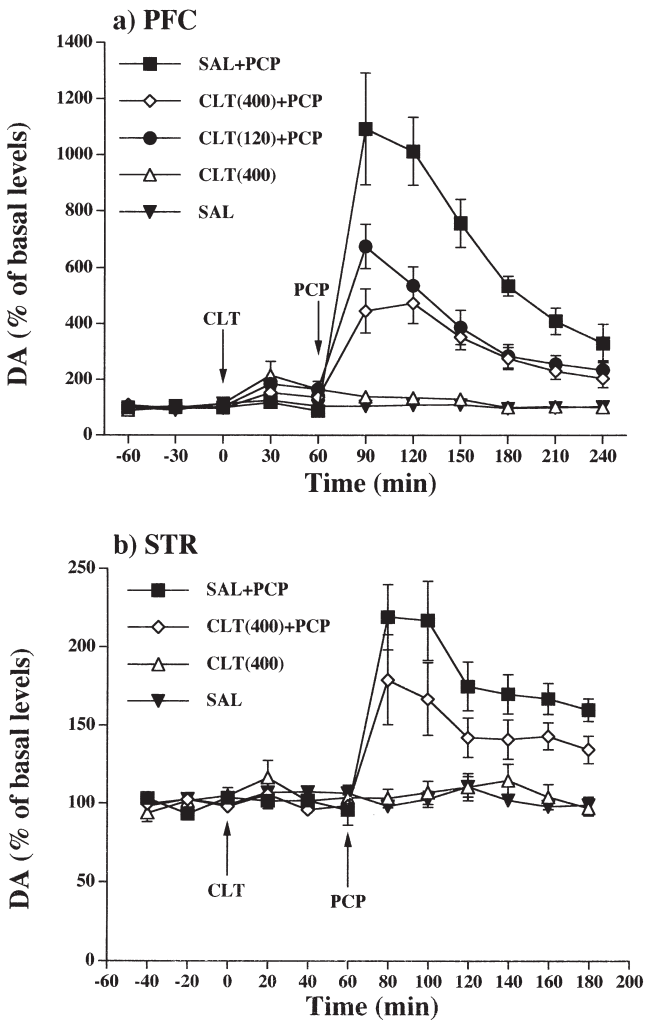


FIG. 1. Effects of pretreatment with ceruletide (CLT; 120 and 400 μg/kg, IP) on phencyclidine (PCP; 5.0 mg/kg, IP)-induced increases in extracellular DA levels in the rat prefrontal cortex (PFC; a) and striatum (STR; b). The rats were pretreated with ceruletide or saline 60 min prior to administration of PCP or saline. Each point represents the mean ± SEM ($n$ = 4–7 for each treatment group) of corresponding time points, expressed as percentages of the predrug basal level. When administered alone, ceruletide (400 μg/kg, IP) caused a significant increase in basal extracellular DA levels in the prefrontal cortex during the first 30-min period after injection, but had no significant effect on basal extracellular DA levels in the striatum. PCP produced a greater increase in extracellular DA levels in the prefrontal cortex than in the striatum. Two-way repeated measures ANOVA with post hoc comparison revealed pretreatment with ceruletide (120 and 400 μg/kg, IP) to significantly attenuate the PCP-induced increase in extracellular DA levels in the prefrontal cortex. However, repeated measures ANOVA failed to demonstrate that ceruletide affected the PCP-induced increase in extracellular DA levels in the striatum.

*Effects of Ceruletide on Basal and PCP-Induced Increases in Extracellular DA Levels in the Prefrontal Cortex (Fig. 1a) and the Striatum (Fig. 1b)*

When administered alone, ceruletide (400 μg/kg, IP) significantly increased basal extracellular DA levels in the prefrontal cortex (245 ± 52%) during the first 30-min period after injection, compared to the saline control. Two-way re-

peated measures ANOVA revealed a significant effect of treatment, $F(1, 7) = 7.41$, $p = 0.029$, and a significant interaction between treatment and time, $F(5, 35) = 4.39$, $p = 0.003$, in the prefrontal cortex. Ceruletide (400 μg/kg, IP) alone had no significant effect on basal extracellular DA levels in the striatum.

Pretreatment with ceruletide, administered 60 min prior to PCP, significantly attenuated the effect of PCP on extracellular DA levels in the prefrontal cortex. Two-way repeated measures ANOVA revealed a significant dose effect, $F(2, 13) = 14.93$, $p < 0.001$, and a significant interaction between the dose and time, $F(14, 91) = 5.18$, $p < 0.001$. Post hoc comparison indicated that both 120 and 400 μg/kg (IP) of ceruletide significantly decreased the PCP-induced increase in extracellular DA levels up to 676 ± 78% and 473 ± 72% of the predrug basal levels ($p < 0.001$), respectively. Ceruletide (400 μg/kg, IP) pretreatment had no significant effect on the PCP-induced increase in extracellular DA levels in the striatum (182 ± 28%; $p > 0.1$).

*Effects of Ceruletide on Basal and PCP-Induced Changes in Extracellular 5-HT Levels in the Prefrontal Cortex (Fig. 2a) and the Striatum (Fig. 2b).*

When administered alone, ceruletide (400 μg/kg, IP) did not affect basal extracellular 5-HT levels in either the prefrontal cortex or the striatum, compared to the saline control. Pretreatment with ceruletide significantly attenuated the effect of PCP on extracellular 5-HT levels in the prefrontal cortex. Repeated measures ANOVA revealed a significant dose effect, $F(2, 13) = 4.10$, $p = 0.042$, but no significant interaction between dose and time, $F(14, 91) = 1.50$, $p > 0.1$. Post hoc comparison indicated that both 120 and 400 μg/kg (IP) of ceruletide significantly decreased the PCP-induced increase in extracellular 5-HT levels in the prefrontal cortex up to 154 ± 17 and 120 ± 11% of the predrug basal levels ($p = 0.041$ and $p = 0.019$), respectively.

PCP did not affect extracellular 5-HT levels in the striatum of the rats pretreated with either ceruletide or saline.

*Effects of the Selective CCK Receptor Antagonist Plus Ceruletide on PCP-Induced Increases in Extracellular DA (Fig. 3a) and 5-HT (Fig. 3b) Levels in the Prefrontal Cortex*

Lorglumide (1.0 mg/kg, SC), a selective CCK-A receptor antagonist, administered 5 min prior to ceruletide, did not influence the effect of ceruletide on the PCP-induced increase in extracellular DA and 5-HT levels in the prefrontal cortex. There was no significant difference between the ceruletide-PCP group and the lorglumide–ceruletide–PCP group for DA, $F(1, 8) = 1.38$, $p = 0.274$, and 5-HT, $F(1, 8) = 3.64$, $p = 0.093$, respectively.

PD 135,158 (0.1 mg/kg, SC), a selective CCK-B receptor antagonist, administered 5 min prior to ceruletide, reversed the effect of ceruletide to inhibit the PCP-induced increase in extracellular DA and 5-HT levels in the prefrontal cortex. The group of rats receiving PD 135,158 and ceruletide rather showed a trend for potentiation of the PCP-induced increase in extracellular DA levels [2,190 ± 343%; $F(1, 6) = 4.42$, $p = 0.080$]. There was also significant difference in the time-dependent effects on extracellular 5-HT levels between the saline-PCP group and the PD 135,158–ceruletide–PCP group [165 ± 21% vs. 293 ± 41%; $F(1, 8) = 27.75$, $p < 0.001$].

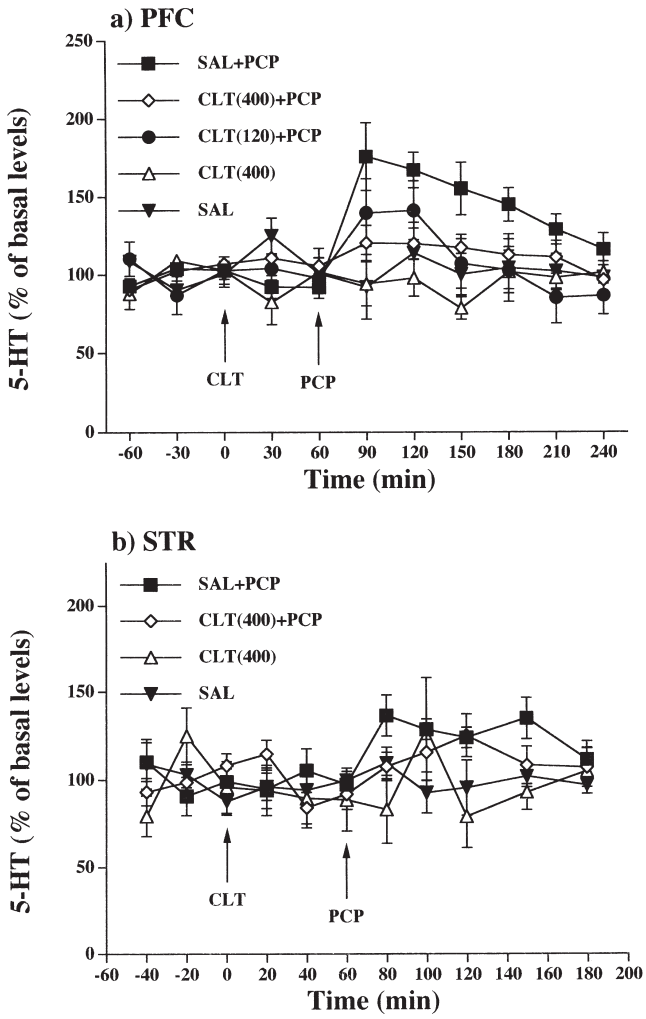


FIG. 2. Effects of pretreatment with ceruletide (CLT; 120 and 400 μg/kg, IP) on phencyclidine (PCP; 5.0 mg/kg, IP)-induced changes in extracellular 5-HT levels in the rat prefrontal cortex (PFC; a) and the striatum (STR; b). The rats were pretreated with either ceruletide or saline 60 min prior to administration of PCP or saline. Each point represents the mean ± SEM ($n$ = 4–7 for each treatment group) of corresponding time points, expressed as percentages of the predrug basal level. When administered alone, ceruletide (400 μg/kg, IP) had no significant effect on basal extracellular 5-HT levels in either the prefrontal cortex or the striatum. PCP caused a significant increase in extracellular 5-HT levels in the prefrontal cortex but not in the striatum. Two-way repeated measures ANOVA with post hoc comparison showed pretreatment with ceruletide (400 and 120 μg/kg, IP) to significantly attenuate the PCP-induced increase in extracellular 5-HT levels in the prefrontal cortex.

*Effects of Ceruletide on MK-801–Induced Changes in Extracellular DA (Fig. 4a) and 5-HT (Fig. 4b) Levels in the Prefrontal Cortex*

Pretreatment with ceruletide (400 μg/kg IP) had no significant effect on the MK-801–induced increase in extracellular DA levels in the prefrontal cortex (301 ± 127%). MK-801 did not affect extracellular 5-HT levels in the prefrontal cortex of rats pretreated with either ceruletide or saline.

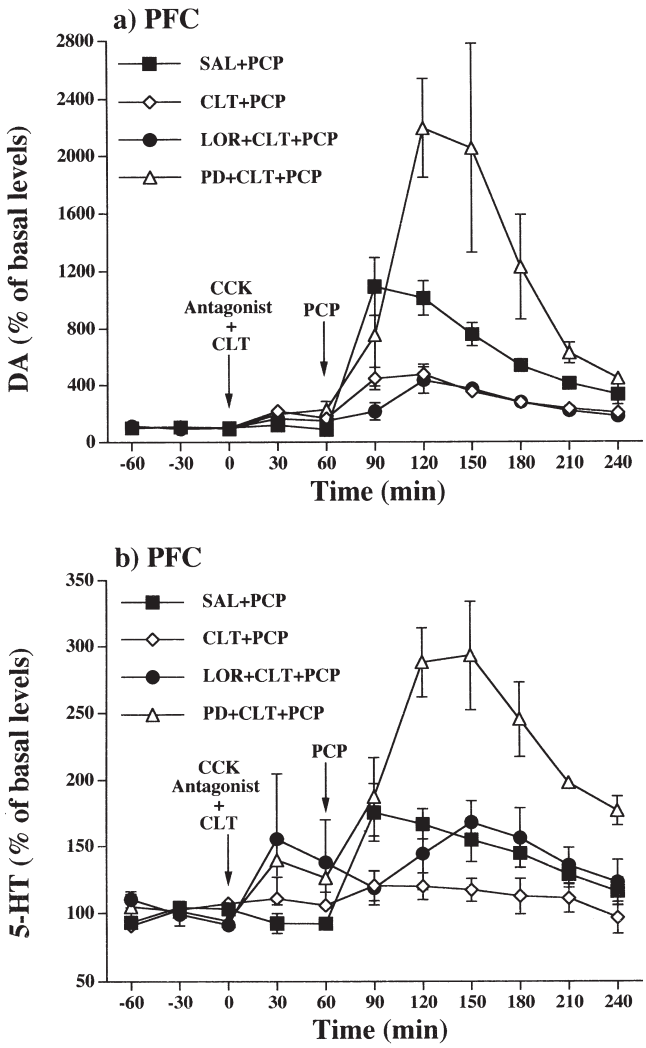


FIG. 3. Effects of pretreatment with lorglumide (LOR; 1.0 mg/kg, SC) or PD 135,158 (PD; 0.1 mg/kg, SC), and ceruletide (CLT; 400 μg/kg, IP) on phencyclidine (PCP; 5.0 mg/kg, IP)-induced increases in extracellular DA (a) and 5-HT (b) levels in the rat prefrontal cortex (PFC). The rats were pretreated with lorglumide or PD 135,158 prior to 5 min ceruletide, and then were injected with PCP 60 min after ceruletide. The data are mean ± SEM ($n$ = 4–6 for each treatment group) of corresponding time points, expressed as percentages of the predrug basal level. Repeated measures ANOVA revealed PD 135,158 to reverse the effect of ceruletide on the PCP-induced increase in extracellular DA and 5-HT levels in the prefrontal cortex. The PD 135,158–ceruletide–PCP group showed a greater increase in extracellular DA and 5-HT levels following PCP than the saline–PCP group. Lorglumide was less effective to reverse the effect of ceruletide on the PCP-induced increase in extracellular DA and 5-HT levels.

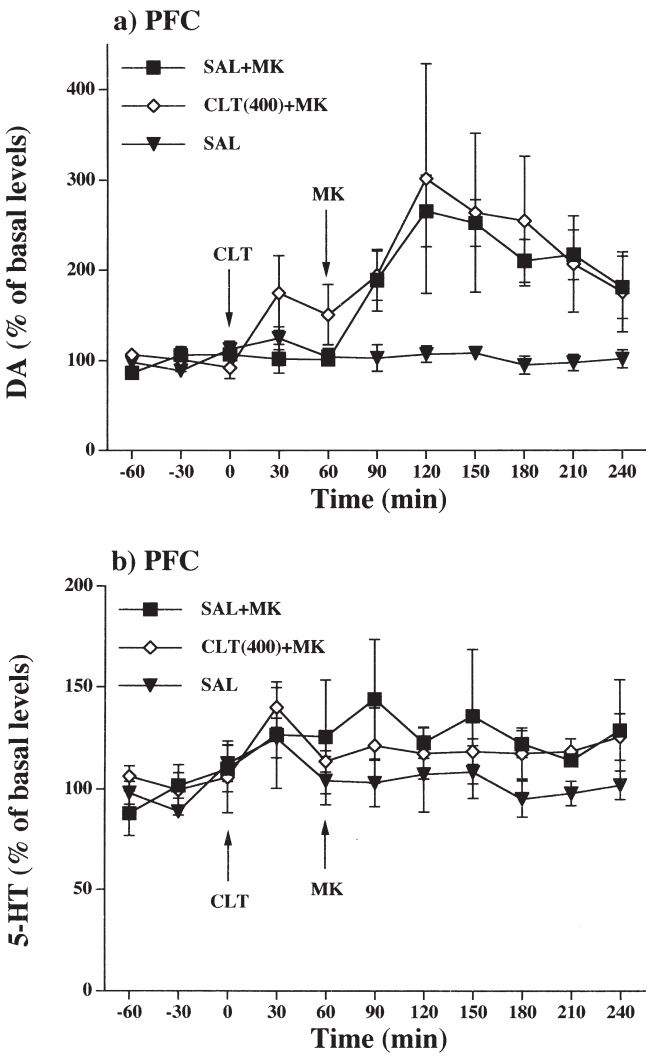


FIG. 4. Effects of pretreatment with ceruletide (CLT; 400 μg/kg, IP) on dizocilpine (MK-801; 0.5 mg/kg, IP)-induced changes in extracellular DA (a) and 5-HT (b) levels in the rat prefrontal cortex (PFC). The rats were pretreated with ceruletide or saline 60 min prior to administration of MK-801 or saline. The data are mean ± SEM ($n$ = 4–7 for each treatment group) of corresponding time points, expressed as percentages of the predrug basal level. MK-801 produced a significant increase in extracellular DA levels but had no significant effect on extracellular 5-HT levels in the prefrontal cortex. Repeated-measures ANOVA failed to show that pretreatment with ceruletide had any significant effect on the MK-801–induced increase in extracellular DA levels in the prefrontal cortex.

## DISCUSSION

The most remarkable finding of this study was that CCK-related decapeptide ceruletide attenuated PCP-induced release of DA and 5-HT in the prefrontal cortex, but not in the striatum. When administered alone, ceruletide increased basal DA release in the prefrontal cortex, but not in the striatum. These results thus suggest that ceruletide may differentially modulate basal and PCP-induced release of DA and 5-HT in a region-specific manner.

In vivo microdialysis studies have demonstrated that CCK-8-S and ceruletide modulate basal DA release in a region-specific manner. The present finding is consistent with the previous report by others that systemically administered ceruletide increased basal DA release in the medial prefrontal cortex (10). In contrast with the results reported here, Hamamura et al. (11) reported that ceruletide decreases basal DA release in the striatum, although others reported that it has no effect in

the striatum (21). The regional effect of ceruletide on basal DA release thus appears ambiguous. This discrepancy may be due to multiple sites and routes of action of ceruletide. Ceruletide, like CCK-8-S, binds nonspecifically to both CCK-A and CCK-B receptor subtypes (27,44). CCK-B receptors are widely distributed throughout the brain, while CCK-A receptors are localized in the more restricted brain regions such as the nucleus tractus solitarius, area postrema, dorsal raphe nucleus, interpeduncular nucleus, and striatum (44). Ceruletide-induced increases in basal DA release in the prefrontal cortex may be mediated in part by the stimulation of CCK-A receptors, because L 364,718, a selective CCK-A receptor antagonist, antagonized the effect of ceruletide on extracellular DA metabolite levels in the prefrontal cortex (10). CCK-8-S may also produce an increase in the firing rate of most of DA neurons in the ventral tegmental area (VTA) and in DA release in the terminal regions by the stimulation of CCK-A receptors (12,19,20,39). Accordingly, ceruletide may thus enhance the neuronal activity of DA neurons in the VTA via CCK-A receptors to increase basal DA release in the prefrontal cortex. However, the vagotomy has been reported to attenuate the effects of ceruletide on basal DA release (10,11), leaving open the possibility that systemically administered ceruletide may primarily act on the vagal afferent nerves in visceral organs to exert its effects in the CNS.

The present study demonstrated that PD 135,158, a selective CCK-B receptor antagonist, but not lorglumide, a selective CCK-A receptor antagonist, reverses the inhibitory effect of ceruletide on PCP-induced DA release in the prefrontal cortex. This suggests that ceruletide may inhibit PCP-induced DA release in the prefrontal cortex via CCK-B receptors, whereas its effect on basal prefrontal cortex DA release is likely due to the stimulation of CCK-A receptors (10). It should be noted that PD 135,158 appears to potentiate PCP-induced DA release (Fig. 3a). Hagino and Moroji (10) reported that L 365,260, a selective CCK-B receptor antagonist, has no effect on basal extracellular DA metabolite levels in the prefrontal cortex. The stimulation of CCK-B receptors may thus play an inhibitory role in not basal but evoked DA release in the prefrontal cortex. Although our study showed that ceruletide had no effect on PCP-induced DA release in the striatum, CCK-8-S and ceruletide have been demonstrated to inhibit amphetamine or haloperidol-induced DA release in the striatum and nucleus accumbens by the stimulation of CCK-B receptors (2,21,22). It is, therefore, likely that systemically administered ceruletide could enter the brain through the blood–brain barrier and directly act on CCK-B receptors.

It should be noted that, although the dose of dizocilpine (MK-801) would be equivalent to that of PCP for the antagonism of NMDA receptors (41), PCP increased extracellular DA levels in the prefrontal cortex to a greater extent than MK-801 (Figs. 1a and 4a). This suggests that PCP-induced prefrontal cortex DA release may be due to not only the NMDA receptor-mediated mechanism but also other mechanism(s) such as interactions with dopamine transporters and voltage-dependent $K^+$ channels (17,26). Further studies are needed to elucidate the contribution of all these effects to PCP-induced DA release in the prefrontal cortex. We previously suggested that PCP and MK-801 may facilitate prefrontal DA release, at least in part, by the inhibition of γ-aminobutyric acid (GABA) release from GABA interneurons (48), which tonically inhibit the dopaminergic neurons in the prefrontal cortex (37). GABA and CCK have been demonstrated to coexist in cortical interneurons (18). Furthermore, the effects of CCK-8-S and ceruletide on the release of GABA and excitatory amino acid such as aspartate (Asp) and glutamate (Glu) have been reported (9,33,38), while PCP may reduce $K^+$-induced release of Asp and Glu in the anterior cingulate cortex (46). It could, therefore, be hypothesized that ceruletide might increase the release of Asp, Glu, and/or GABA in the prefrontal cortex and then antagonize the effect of PCP to increase prefrontal cortex DA release via the blockade of NMDA receptors. However, this hypothesis is not consistent with the present results that ceruletide had no effect on MK-801–induced increases in extracellular DA levels in the prefrontal cortex (Fig. 4a). CCK is unlikely to influence the prefrontal cortex DA release induced by the antagonism of NMDA receptors. Interestingly, the inhibitory effect of PCP on $K^+$-induced release of CCK may not be attributable to the NMDA receptor-mediated mechanism (1). Ceruletide might selectively attenuate the inhibition of DA reuptake by PCP, because PCP has been known to have a considerably high affinity for DA transporters (26). However, it seems unlikely, because ceruletide had no effect on PCP-induced DA release in the striatum, where PCP-induced DA release may most likely be due to an interaction with DA transporters (47).

Ceruletide could set a limit of an increase in extracellular DA levels in the prefrontal cortex only when it is excessively enhanced by PCP. It has yet to be examined whether ceruletide could also inhibit prefrontal cortex DA release stimulated by other potent DA releasers such as amphetamine and cocaine. CCK-8-S has been reported to enhance stimulation by endogenous DA of $D_2$ autoreceptors located on the VTA neurons (19), which effect may cause a decrease in the releasable pool of DA available for PCP-induced release. Such a potentiation by ceruletide of $D_2$ autoreceptor-mediated regulation may in part account for the inhibitory effect of ceruletide on the PCP-induced DA release. However, this interaction in the VTA has been suggested to be mediated by CCK-A receptors (12,19,20). Other factors not yet apparent may also be involved.

Dopaminergic systems in the prefrontal cortex have been suggested to play an important role in the cognitive functions. Although the hypofunction of the prefrontal cortex DA neurons has been postulated to be the basis for the negative symptoms of schizophrenia (6), an excessive dopaminergic activity in the prefrontal cortex may also impair the cognitive functions (29). There may thus be a critical range of prefrontal cortex dopaminergic activity for the normal cognitive functions. If so, the differential effects of ceruletide on basal and PCP-induced DA release, for example, the stimulation of basal levels and the inhibition of evoked levels, may be useful for the modulation of prefrontal cortex dopaminergic activity to the optimal range. Therefore, ceruletide would be expected to be effective on various cognitive disorders such as schizophrenia, attention deficit disorder, and dementia.

It is also noteworthy that ceruletide attenuated PCP-induced 5-HT release, but had no effect on basal 5-HT release, in the prefrontal cortex. Although it is unclear whether this effect of ceruletide is similar to the effect of ceruletide on PCP-induced DA release, it may also be mediated by the stimulation of CCK-B receptors. Despite the pretreatment with ceruletide, PD 135,158 potentiated PCP-induced prefrontal cortex 5-HT release (Fig. 3b). Lorglumide was less effective to reverse the effect of ceruletide. These results suggest that the stimulation of CCK-B receptors may inhibit the evoked release of 5-HT as well as DA. However, this hypothesis is not consistent with the report that L 365,260, a selective CCK-B receptor antagonist, blocked prefrontal cortex 5-HT

release induced by BOC-CCK-4, a CCK-B receptor agonist, in guinea pigs on exposure to the X-maze (36). The inhibitory effect of ceruletide on PCP-induced 5-HT release in the prefrontal cortex may also have a clinical significance for a certain aspect of the emotional and cognitive functions. Both 5-HT and CCK neurons in the prefrontal cortex have been suggested to be involved in the anxiogenic mechanism(s) (4, 35,36). CCK-4 has been demonstrated to produce anxious behavior and enhance the evoked 5-HT release in animal models of anxiety (36), while the CCK-B receptor antagonist has been reported to have an anxiolytic effect (4). The present results may imply a modulatory effect of ceruletide on anxiety, yet it remains to be determined whether ceruletide, an analogue of CCK-8 but not CCK-4, has an anxiogenic or anxiolytic effect.

In summary, pretreatment with ceruletide attenuated PCP-induced release of DA and 5-HT in the rat prefrontal cortex, while ceruletide alone increased basal prefrontal cortex DA release. The inhibitory effects of ceruletide on PCP-induced DA and 5-HT release were reversed by the CCK-B receptor antagonist. These results suggest that ceruletide may differentially modulate basal and PCP-induced release of DA and 5-HT in the prefrontal cortex by the stimulation of different subtypes of CCK receptors. However, it seems unlikely that an interaction between CCK and amino acidergic neurons would account for the inhibitory effect of ceruletide on PCP-induced DA release.

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