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Barrel Rotation in Rats Induced by SMS 201-995: Suppression by Ceruletide

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ISHIKAWA, Y., A. SHIMATSU, Y. MURAKAMI AND H. IMURA. *Barrel rotation in rats induced by SMS 201-995: Suppression by ceruletide*. PHARMACOL BIOCHEM BEHAV **37**(3) 523–526, 1990.—Intracerebroventricular administration of SMS 201-995 (5 μg/rat), a somatostatin analogue, induced barrel rotation in rats. Pretreatment with ceruletide (40 μg/100 g b.wt., IP) 3 days or 7 days prior to the injection of SMS 201-995 significantly inhibited the response rate of barrel rotation induced by SMS 201–995, but not that induced by arginine-vasopressin (1 μg/rat, ICV). The suppressive effect of ceruletide on barrel rotation could be partially countered by MK-329, a selective peripheral CCK (CCK-A) receptor antagonist. Desulfated cerulein did not affect the barrel rotation induced by SMS 201-995. These findings suggest that ceruletide specifically suppresses the barrel rotation evoked by SMS 201-995 in a long-lasting manner possibly acting through CCK-A receptor.

Somatostatin SMS 201-995 Barrel rotation Ceruletide MK-329 CCK-A receptor

INTRACEREBROVENTRICULAR (ICV) injection of somatostatin induces sustained, twisting posture followed by repetitive lateral rotations along the longitudinal axis, termed barrel rotation (BR) (2, 4, 6). BR has also been reported to be induced by vasopressin (2,13), cholecystokinin (27–33) (16), bradykinin antagonists (20) and chlorpromazine-methiodide (5), an antimuscarinic agent. However, the neuroanatomical and pharmacological basis for BR remain to be determined. The decapeptide from the frog, cerulein (ceruletide, CER), is chemically closely related to the C-terminal octapeptide cholecystokinin (CCK-8). Like CCK-8, CER produces many behavioral effects in mammals: inhibition of intake of food and water; antinociception; sedation; catalepsy; ptosis; antistereotypic, anticonvulsive, and tremorolytic effects; inhibition of self-stimulation (24). Recently it has been reported that CER and CCK suppress tardive dyskinesia in humans (19) and in experimental animals (23). In the present study, we examined the behavioral effect of SMS 201-995 [D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol)], a somatostatin analogue (3), and reported that CER suppresses BR evoked by SMS 201-995.

METHOD

Animals

Male Wistar strain rats (Japan Animal Co., Osaka, Japan), weighing 300–400 g, were maintained on a 12-hour light-dark cycle and given laboratory chow and tap water ad lib. Each animal was used only once.

Drugs

SMS 201-995 was kindly supplied by Sandoz Co., Ltd. (Tokyo, Japan). Ceruletide (cerulein diethylammonium hydrate), desulfated cerulein, and MK-329 [=L-364,718; 3s-(−)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3yl)-1H-indole-2-carboxamide; Merck Sharp & Dohme] (7) were kindly supplied by Shionogi Research Laboratories (Osaka, Japan). Other drugs used were somatostatin (Protein Research Institute, Osaka, Japan), arginine-vasopressin (Protein Research Institute), atropine sulfate (E. Merck, Darmstadt), hexamethonium bromide (Tokyo Kasei Co., Tokyo), and physostigmine sulfate (Nakarai Chemical Co., Kyoto). The drugs were dissolved in physiological saline. MK-329 was first dissolved in dimethyl sulfoxide (DMSO) and further diluted with saline to the final concentration of DMSO at 10%.

Procedure

The animals were anesthetized with chloral hydrate (3.5 mg/100 g b.wt., IP) and a polyethylene catheter (PE 10) was implanted into the right cerebral ventricle using a stereotaxic apparatus, 3 to 5 days before the experiment as previously

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TABLE 1

THE RESPONSE RATE OF BARREL ROTATION INDUCED BY SOMATOSTATIN AND SMS 201-995

Treatment	Dose (μg/rat)	Barrel Rotation
Saline		0/8
Somatostatin	1	0/5
	5	2/11
	50	8/10*
SMS 201-995	0.5	0/9
	5	13/18*

Response is expressed as number of animals with rotation/number of animals injected.

*$p<0.01$ vs. saline (by Fisher's exact probability test).

TABLE 2

THE EFFECT OF CERULETIDE (40 μg/100 g b.wt., IP, 3 DAYS BEFORE) ON ARGININE-VASOPRESSIN (ICV) INDUCED BARREL ROTATION IN RATS

Treatment	Dose (μg/rat)	Barrel Rotation
Saline		0/8
Arginine-vasopressin	0.2	4/12
Arginine-vasopressin	1.0	4/10
Ceruletide + Arginine-vasopressin	1.0	4/9

Response is expressed as number of animals with rotation/number of animals injected.

described (1,12). On the day of experiment, the animals were fasted for 2 hours. Physiological saline, SMS 201-995 (0.5, 5 μg), somatostatin (5, 50 μg) or arginine-vasopressin (0.2, 1.0 μg) were injected ICV in a volume of 10 μl over a period of 60 seconds through the catheter. The animals were pretreated with CER (1.6, 8, 40 μg/100 g b.wt.) intraperitoneally (IP) in a volume of 0.1 ml/100 g b.wt. 30 minutes, 3, 7 or 14 days prior to the injection of SMS 201-995 (5 μg/rat) or arginine-vasopressin (1.0 μg). Desulfated cerulein (40 μg/100 g b.wt.) was administered IP 3 days prior to injection of SMS 201-995. MK-329 (400 μg/100 g b.wt.), or vehicle (10% DMSO in saline) was injected IP 20 min before the administration of CER (40 μg/100 g b.wt.). In another experiment, CER (1.6 or 8 μg/10 μl/rat), atropine sulfate (30 μg/rat) or hexamethonium bromide (40 μg/rat) were administered ICV with SMS 201-995 (5 μg/rat). Physostigmine sulfate (50 μg/100 g b.wt.) or saline was administered IP 30 min before the injection of SMS 201-995 (5 μg/rat) in animals pretreated with CER (40 μg/100 g b.wt., IP, 3 day). The behavioral changes were observed for 60 min after the injection of SMS 201-995. Fisher's exact probability test was used for the statistical analysis.

RESULTS

ICV administration of somatostatin (5, 50 μg/rat), SMS 201-995 (5 μg/rat) and arginine-vasopressin (0.2, 1 μg/rat) evoked BR in rats (Tables 1 and 2). The mean (±SE) number of BRs induced by SMS 201-995 (5 μg/rat) was 12 (±4). Pretreatment of the animals with CER (40 μg/100 g b.wt., IP) 3 days prior to the injection of SMS 201-995 significantly inhibited the response of BR induced by SMS 201-995, but not that induced by arginine-vasopressin, as shown in Fig. 1a and Table 2. The suppression of BR by CER was evident 3 and 7 days after the injection of CER (40 μg/100 g b.wt., IP). BR induced by SMS 201-995 was no longer suppressed after 14 days after the injection of CER (Fig. 1b).

As shown in Fig. 2, desulfated cerulein (dCER; 40 μg/100 g b.wt., IP) or MK-329, a selective peripheral CCK (CCK-A) receptor antagonist (400 μg/100 g b.wt., IP), did not affect the BR induced by SMS 201-995. However, MK-329 did partially antagonize the inhibition by CER of SMS 201-995-induced BR. The ICV administration of CER (1.6, 8 μg/rat) with SMS 201-995 did not inhibit the response of BR (Fig. 2).

Since somatostatin has been reported to increase acetylcholine turnover in brain regions, we investigated the effect of cholinergic drugs on SMS 201-995-induced BR in rats. Figure 3 shows that simultaneous administration of atropine sulfate (30 μg/rat), a muscarinic cholinergic receptor-blocker, suppressed the BR induced by SMS 201-995 (5 μg/rat), but hexamethonium (40 μg/rat), a nicotinic receptor-blocker, did not suppress the BR. Pretreatment of the animals with physostigmine sulfate (50 μg/100 g b.wt., IP), a cholinesterase inhibitor, did not potentiate the response of BR induced by SMS 201-995 nor antagonized the inhibition of CER on BR.

DISCUSSION

The present study has demonstrated that SMS 210-995 induced BR in a smaller dose than that of the "native" somatostatin. Since the ICV administration of larger doses of somatostatin caused lethal effects in rats [(2) and our unpublished observation], we preferred using a somatostatin analogue, SMS 201-995, at the dose of 5 μg/rat, which was not lethal, in the following experiments. SMS 201-995 is more potent and less susceptible to degradation than "native" somatostatin (3).

The present study has also demonstrated that the pretreatment with CER inhibited the response rate of BR induced by SMS 201-995 in a dose-related manner. It has no effect on the BR induced by arginine-vasopressin, suggesting the selectivity of CER on SMS 201-995-induced BR. Pharmacological (7), electrophysiological (10) and binding (18) studies describe the existence of two types of CCK receptors: the peripheral-type CCK (CCK-A) receptor which recognizes sulfated CCK-8 and is blocked by MK-329 (7) and the brain-type CCK (CCK-B) receptor which also recognized smaller fragments of CCK, such as CCK-4. Desulfated cerulein, which had higher affinity to the CCK-B receptor than to the CCK-A receptor (11,18), did not inhibit SMS 201-995-induced BR. MK-329 partially antagonized the inhibition by CER of SMS 201-995-induced BR. These findings suggest that the inhibitory effect of CER is mainly mediated through the CCK-A receptor (22). The site of action of CER remains to be determined. CNS administration of CER at two doses did not inhibit the BR evoked by SMS 201-995. CER has been shown to have various CNS effects that arise through vagal afferents (8,24). It is possible, therefore, that CER is acting peripherally through CCK-A receptor to inhibit the SMS 201-995-induced BR.

CNS administration of atropine, but not hexamethonium, suppressed BR induced by SMS 201-995, which suggests that central muscarinic cholinergic mechanisms are involved in the SMS 201-995-induced BR (6). Somatostatin has been shown to increase acetylcholine turnover in the brain (15). CCK-8 has also been shown to modulate the release of acetylcholine from the cerebral cortex in a biphasic manner; acetylcholine release was

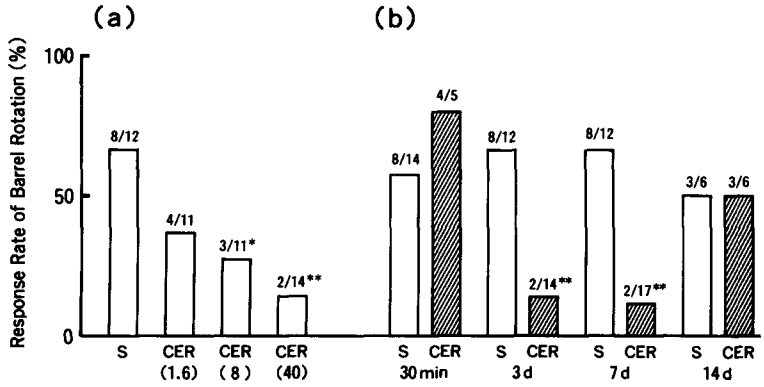

FIG. 1. Effects of ceruletide on SMS 201-995-induced barrel rotation in rats. (a) Ceruletide (CER; 1.6, 8 or 40 μg/100 g b.wt.) or saline (S) was injected IP 3 days prior to the injection of SMS 201-995 (5 μg/rat, ICV). Response is expressed as number of animals with rotation/number of animals injected. *$p=0.07$, **$p<0.01$ vs. saline (by Fisher's exact probability test); (b) Ceruletide (CER; 40 μg/100 g b.wt.) or saline (S) was injected IP, 30 min, 3, 7 or 14 days prior to the injection of SMS 201-995 (5 μg/rat, ICV). **$p<0.01$ vs. corresponding control saline-injected group.

enhanced by smaller doses of CCK-8, whereas larger doses of CCK-8 decreased acetylcholine turnover (14). We, therefore, investigated whether the cholinergic mechanism might be involved in the action of CER. However, the enhancement of endogenous cholinergic tone by physostigmine neither enhanced the SMS 201-995-induced BR, nor modified the suppressive effect of CER on BR.

It is noteworthy that CER suppressed BR induced by SMS 201-995 for 7 days. The similar long-lasting effect of CER on the central nervous system has previously been reported (17) following one single dose of either CCK-8 or CER. ^{3}H-Spiperone binding in both the striatum and the nucleus accumbens was increased for a period up to two weeks (9) after combined administration of CER with haloperidol. Antagonism of amphetamine-induced hyperactivity was observed for two weeks (17). These findings are in good agreement with the clinical observation by Nishikawa *et al.* (19) of long-lasting effect of CER on tardive dyskinesia. Although the mechanism of long-lasting effect of CER is unclear, SMS 201-995-induced BR may be a useful model for studying the mechanisms by which CER inhibits human movement disorders.

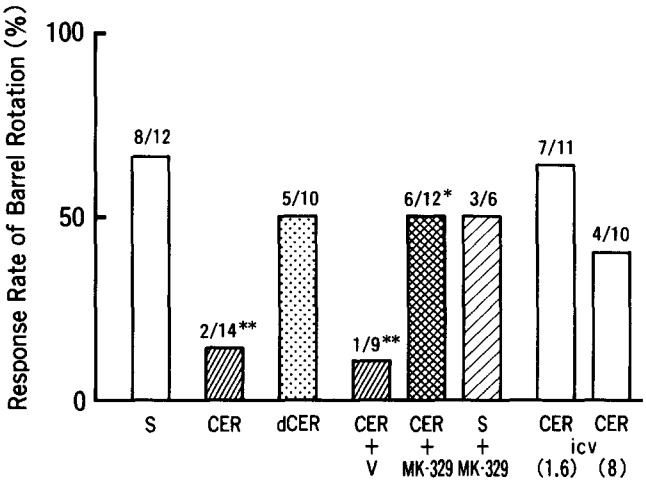

FIG. 2. Effects of desulfated cerulein, MK-329 and ceruletide on SMS 201-995-induced barrel rotation in rats. Ceruletide (CER; 40 μg/100 g b.wt.) or desulfated cerulein (dCER; 40 μg/100 g b.wt.) was injected IP 3 days prior to the injection of SMS 201-995 (5 μg/rat, ICV). Vehicle (10% DMSO in saline) or MK-329 (400 μg/100 g b.wt.) was injected IP 30 min before the injection of CER (40 μg/100 g b.wt.). In another experiment, CER (1.6 or 8 μg/rat) was injected ICV simultaneously with SMS 201-995. **$p<0.01$ vs. saline control group. *$p=0.07$ vs. CER+V group.

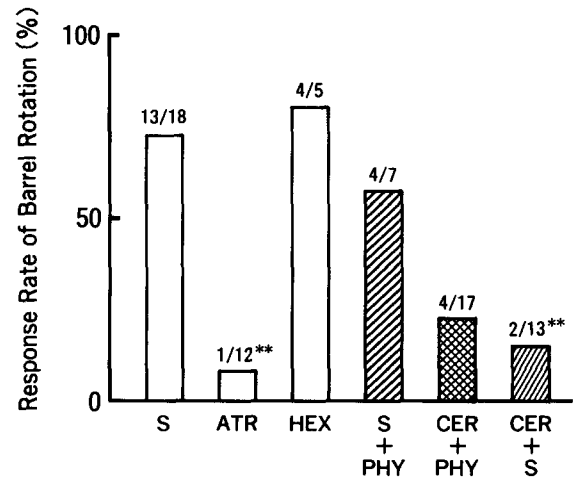

FIG. 3. Effects of cholinergic drugs on SMS 201-995-induced barrel rotation in rats. Atropine sulfate (ATR; 40 μg/rat) or hexamethonium bromide (HEX; 50 μg/rat) was injected ICV simultaneously with SMS 201-995 (5 μg/rat, ICV). Physostigmine sulfate (PHY; 50 μg/100 g b.wt.) or saline (S) was injected IP 30 min before the injection of SMS 201-995. Ceruletide (CER; 40 μg/100 g b.wt.) was injected IP 3 days before the injection of SMS 201-995. **$p<0.01$ vs. saline group.

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