Sedative Action of Cholecystokinin Octapeptide on Behavioral Excitation by Thyrotropin Releasing Hormone and Methamphetamine in the Rat

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Abstract Intracerebroventricular (i.c.v.) injection of C-terminal octapeptide of cholecystokinin (CCK-8) in rats prolonged pentobarbital- and ethanol-induced sleeping time, but non-sulfated CCK-8 (CCK-8-NS) had no effect and caerulein showed a tendency to prolong the pentobarbital narcosis. On the other hand, i.c.v. injection of thyrotropin releasing hormone (TRH) shortened the sleeping time and the effect of CCK-8 was apparently antagonized by combined administration of TRH. Spontaneous locomotor activity in the late morning and early afternoon was not affected by CCK-8, but it increased following i.c.v. injection of CCK-8-NS. Hyperactivity produced by TRH and methamphetamine was suppressed by i.c.v. injection of CCK-8, while CCK-8-NS showed a tendency to enhance the methamphetamine-induced hyperactivity and caerulein had no effect. These results indicate that CCK-8 has a sedative action and antagonizes the behavioral excitation caused by TRH and methamphetamine, but that the effects of CCK-8-NS and caerulein were rather the opposite of those of CCK-8. In an additional experiment the TRH-induced body shaking response was not affected by combined administration of CCK-8.

Key Words: cholecystokinin, thyrotropin-releasing hormone, sleeping time, locomotor activity.

Recently, it has been reported by ZETLER (1980a) that s.c. injection of caerulein and CCK-8 delays the onset and retards the development of toxic effects of several convulsants and increases the seizure threshold of i.v. injection of these drugs. ZETLER (1980b) also found that s.c. injection of caerulein and CCK-8 inhibited spontaneous rearing activity and prolonged hexobarbital sleeping time in mice. On the other hand, TRH has been shown to potentiate L-DOPA-induced excitation (PLOTNIKOFF *et al.*, 1972, 1974) and to shorten the sleeping time induced by barbiturates and other depressants (BRESE *et al.*, 1975; BROWN and VALE, 1975; COTT *et al.*, 1976) in rats and mice. Central behavioral excita-

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tion and a hyperthermic response to TRH were also reported in rabbits (CARINO *et al.*, 1976). Such general excitation included an increase in locomotor activity (MANBERG *et al.*, 1979). According to these literature data, CCK-8 is likely to antagonize the behavioral excitation induced by TRH and other central stimulants. The present study was carried out to ascertain this possibility, comparing the effects of CCK-8 with those of its related peptides.

MATERIALS AND METHODS

Male Wistar rats, weighing approximately 250 g, were housed under constant conditions of 25°C and a 12 hr light/dark cycle, with rat biscuits (Oriental Yeast Co.) and water provided *ad libitum*.

The method of i.c.v. injection has been described in detail in our previous paper (ITOH *et al.*, 1979). In brief, a stainless steel guide cannula was fixed stereotaxically on the skull by dental cement, and the animals were allowed 5 days for recovery from the surgery. Thereafter, they became accustomed to insertion of an injection cannula into the left lateral ventricle for another 5 days.

In order to observe effects on the sleeping time, sodium pentobarbital (Pitman-Moore) in a dose of 50 mg/kg was injected i.p. at around 10:00 hr and 10 min later a 5 μ l sample of saline, CCK-8 (Squibb), CCK-8-NS (Peninsula Labs.), caerulein (Calbiochem) or TRH (Sigma) was injected i.c.v. in the doses shown in the following tables. Sleeping time was assessed by observing the time elapsed from the loss of the righting reflex to its recovery. In the case of ethanol-induced sleeping, 25% ethanol solution in a dose of 3.5 g/kg was injected i.p. at around 10:00 hr, and 10 min later test samples were injected i.c.v. and the onset and duration of sleeping time were measured.

Locomotor activity of the rat was monitored using an Automex (Columbus Instruments) with one rat per cage. In the morning, after the rats were allowed 75 min to become accustomed to the cage, a 5 μ l sample of saline, CCK-8, CCK-8-NS, or TRH was injected i.c.v. and activity was recorded every 10 min for 60 min. Methamphetamine (Dainippon Pharm. Co.) was injected s.c. in a dose of 0.15 mg/kg and 1 min later CCK-8, CCK-8-NS or caerulein was injected i.c.v. into the rats. Body shaking following i.c.v. injection of TRH and/or CCK-8 was also counted for 60 min.

The data were analyzed by Student's *t*-test and significant differences between the means were calculated as *P* values.

RESULTS

Pentobarbital-induced sleeping time

After i.p. injection of pentobarbital, CCK-8 in doses from 200 to 1,600 ng was administered i.c.v. and sleeping time was measured. As shown in Table 1,

Table 1. Effect of intracerebroventricular injection of CCK-8, its related peptides, and TRH on pentobarbital-induced narcosis.

Treatment	Dose (ng)	No. of rats	Sleeping time min (mean $\pm$ S.E.M.)		t-Test
			Onset	Duration	
Saline		11	3 $\pm$ 0.2	57 $\pm$ 1.9	
CCK-8	200	5	3 $\pm$ 0.2	56 $\pm$ 2.7	N.S.
	400	11	3 $\pm$ 0.1	62 $\pm$ 1.6	$P < 0.05$
	800	9	3 $\pm$ 0.1	65 $\pm$ 2.4	$P < 0.05$
	1,600	9	3 $\pm$ 0.1	75 $\pm$ 3.2	$P < 0.001$
Saline		13	3 $\pm$ 0.1	56 $\pm$ 2.4	
CCK-8-NS	400	9	3 $\pm$ 0.2	55 $\pm$ 3.1	N.S.
	800	12	3 $\pm$ 0.2	63 $\pm$ 2.8	N.S.
	1,600	10	3 $\pm$ 0.1	55 $\pm$ 2.9	N.S.
Saline		11	3 $\pm$ 0.2	53 $\pm$ 2.6	
Caerulein	200	16	3 $\pm$ 0.1	54 $\pm$ 1.3	N.S.
	400	7	3 $\pm$ 0.1	63 $\pm$ 2.0	$P < 0.05$
	800	9	3 $\pm$ 0.3	61 $\pm$ 2.3	$P < 0.05$
	1,600	8	3 $\pm$ 0.2	59 $\pm$ 2.9	N.S.
Saline		18	3 $\pm$ 0.1	55 $\pm$ 1.5	
TRH	25	7	3 $\pm$ 0.2	50 $\pm$ 2.1	N.S.
	50	14	3 $\pm$ 0.2	48 $\pm$ 1.9	$P < 0.01$
	100	8	3 $\pm$ 0.2	46 $\pm$ 1.6	$P < 0.001$
	200	12	3 $\pm$ 0.1	45 $\pm$ 1.6	$P < 0.001$
	400	10	3 $\pm$ 0.2	41 $\pm$ 2.9	$P < 0.001$
CCK-8	1,600				$P < 0.01$ vs. CCK-8 alone
+ TRH	200	7	3 $\pm$ 0.2	53 $\pm$ 4.9	N.S. vs. TRH alone
CCK-8	1,600				$P < 0.001$ vs. CCK-8 alone
+ TRH	400	9	3 $\pm$ 0.5	46 $\pm$ 3.0	N.S. vs. TRH alone

Each material was injected in a volume of 5 μ l. N.S.: not significant.

after administration of CCK-8 in doses of more than 400 ng, the duration of sleep was prolonged significantly, this effect being dose-dependent. However, CCK-8-NS did not cause such prolongation of the sleeping time. TRH when injected i.c.v. in doses of more than 50 ng, shortened the sleeping time markedly. When 1,600 ng of CCK-8 was injected together with 200 ng of TRH, the narcotic effect of CCK-8 was diminished by TRH, the sleeping time after combined injection of 1,600 ng CCK-8 and 400 ng TRH being remarkably shorter than the time after CCK-8 alone.

Ethanol-induced sleeping time

Similar results were obtained in rats treated with ethanol (Table 2). In the control group, the duration of sleep increased by i.c.v. injection of CCK-8 in a dose of 1,600 ng, while TRH produced a marked reduction in the sleeping time.

Table 2. Effect of intracerebroventricular injection of CCK-8 and TRH on ethanol-induced narcosis.

Treatment	Dose	No. of rats	Sleeping time min (mean $\pm$ S.E.M.)		<i>t</i> -Test
			Onset	Duration	
Saline		10	5 $\pm$ 0.1	58 $\pm$ 3.8	
CCK-8	200 ng	9	3 $\pm$ 0.1	64 $\pm$ 4.9	N.S. vs. saline
	400	8	4 $\pm$ 0.6	60 $\pm$ 6.3	N.S.
	800	12	4 $\pm$ 0.2	59 $\pm$ 3.6	N.S.
	1,600	10	3 $\pm$ 0.2	71 $\pm$ 4.9	$P < 0.01$
TRH	5 μ g	6	3 $\pm$ 0.2	50 $\pm$ 7.2	N.S.
	10	7	3 $\pm$ 0.3	44 $\pm$ 4.3	$P < 0.05$
	20	9	4 $\pm$ 0.9	37 $\pm$ 3.8	$P < 0.001$
	40	12	5 $\pm$ 0.7	39 $\pm$ 5.1	$P < 0.001$
CCK-8 +	1,600 ng	9	3 $\pm$ 0.2	47 $\pm$ 2.3	$P < 0.001$ vs. CCK-8 alone
TRH	40 μ g				N.S. vs. TRH alone

Each material was injected in a volume of 5 μ l. N.S.: not significant.

Table 3. Effect of intracerebroventricular injection of CCK-8 and CCK-8-NS on spontaneous locomotor activity and the effect of CCK-8 on TRH-induced hyperactivity.

Treatment	Dose (ng)	No. of rats	Locomotor activity Counts/hr (mean $\pm$ S.E.M.)	<i>t</i> -Test
Saline		11	593 $\pm$ 53	
CCK-8	100	12	826 $\pm$ 125	N.S. vs. saline
	200	12	689 $\pm$ 60	N.S.
	400	9	648 $\pm$ 67	N.S.
	800	9	524 $\pm$ 98	N.S.
	1,600	9	502 $\pm$ 79	N.S.
Saline		9	518 $\pm$ 94	
CCK-8-NS	400	7	474 $\pm$ 119	N.S. vs. saline
	800	8	1,078 $\pm$ 173	$P < 0.05$
	1,600	9	1,302 $\pm$ 96	$P < 0.001$
TRH	100	12	1,700 $\pm$ 159	
TRH +	100	12	1,301 $\pm$ 109	N.S. vs. TRH alone
CCK-8	100			
TRH +	100	12	1,164 $\pm$ 113	$P < 0.05$
CCK-8	200			
TRH +	100	9	1,021 $\pm$ 97	$P < 0.01$
CCK-8	400			

Each material was injected in a volume of 5 μ l. N.S.: not significant.

When 40 μ g of TRH was administered together with 1,600 ng of CCK-8, the sleeping time was significantly shorter than that after CCK-8 alone. However, it should be noted herewith that the dose of TRH needed to shorten the duration of ethanol-induced sleep was 50 to 100 times greater than the dose to shorten the pentobarbital-induced sleep.

Spontaneous locomotor activity

Locomotor activity of rats, as measured with an Automex between 11:00 and 14:00 hr, was not affected by i.c.v. injection of CCK-8 in doses from 100 to 1,600 ng (Table 3). On the other hand, i.c.v. injection of CCK-8-NS produced an increase in the activity counts. TRH in a dose of 100 ng produced a pronounced increase in the activity and the counts became nearly 3-fold higher than those of the saline control. When TRH at this dose was injected simultaneously with CCK-8, the increase in locomotor activity was significantly suppressed. A similar result was also obtained in an experiment on methamphetamine-induced hyperactivity (Table 4). In this case, an almost 2-fold increase in the activity counts was induced by methamphetamine. This hyperactivity was greatly reduced by CCK-8 in doses of more than 400 ng.

Differing from the effects of this CCK-8, CCK-8-NS in doses of 400 and 800 ng did not cause any decrease in the activity counts in methamphetamine-treated rats. When the latter peptide was injected i.c.v. in relatively large amounts up to 1,600 ng, the activity increased significantly. Caerulein was found to be ineffective in producing any change in methamphetamine-induced hyperactivity.

Table 4. Effect of intracerebroventricular injection of CCK-8 and its related peptides on methamphetamine-induced hyperactivity.

Treatment	Dose (ng)	No. of rats	Locomotor activity Counts/hr (mean $\pm$ S.E.M.)	t-Test
Saline		13	1,396 $\pm$ 136	
CCK-8	200	7	1,149 $\pm$ 136	N.S. vs. saline
	400	9	873 $\pm$ 148	$P < 0.05$
	800	9	804 $\pm$ 127	$P < 0.01$
Saline		13	1,092 $\pm$ 151	
CCK-8-NS	400	7	902 $\pm$ 144	N.S. vs. saline
	800	11	1,440 $\pm$ 309	N.S.
	1,600	13	1,662 $\pm$ 205	$P < 0.05$
Saline		17	1,220 $\pm$ 82	
Caerulein	50	11	1,268 $\pm$ 185	N.S. vs. saline
	100	11	1,278 $\pm$ 183	N.S.
	200	8	969 $\pm$ 222	N.S.
	400	8	1,290 $\pm$ 227	N.S.
	800	11	1,025 $\pm$ 104	N.S.
	1,600	10	1,159 $\pm$ 115	N.S.

Each material was injected in a volume of 5 μ l. N.S.: not significant.

Table 5. Body shaking response induced by TRH.

Treatment	Dose (ng)	No. of rats	No. of shakes Counts/hr (mean $\pm$ S.E.M.)	t-Test
Saline		5	0 $\pm$ 0.2	
TRH	1	5	6 $\pm$ 0.4	N.S. vs. saline
	5	5	10 $\pm$ 2.5	$P < 0.01$
	25	5	22 $\pm$ 3.5	$P < 0.001$
	100	5	55 $\pm$ 5.4	$P < 0.001$
	400	8	76 $\pm$ 6.6	$P < 0.001$
	1,600	5	105 $\pm$ 11.8	$P < 0.001$
CCK-8	100	5	0 $\pm$ 0.2	N.S. vs. saline
	400	5	0 $\pm$ 0.2	N.S.
	1,600	5	4 $\pm$ 0.2	N.S.
TRH + CCK-8	400 1,600	9	86 $\pm$ 4.4	N.S. vs. TRH alone
TRH + CCK-8	1,600 1,600	5	114 $\pm$ 8.7	N.S.

Each material was injected in a volume of 10 μ l. N.S.: not significant.

Body shaking response

The i.c.v. injection of TRH in small doses of even 5 ng caused a peculiar body shaking response, the number of shakes being dose-dependent (Table 5). CCK-8 did not produce such symptoms and the TRH-induced shaking response was not affected by combined administration of CCK-8.

DISCUSSION

TRH has been reported to shorten the sleeping time induced by barbiturates and other depressants, as mentioned before. In pentobarbital-narcotized rabbits, intracerebral injection of TRH was also shown to exert analeptic activity regardless of the brain area injected (CARINO *et al.*, 1976). On the other hand, ZETLER (1980c) found that s.c. injection of caerulein and CCK-8 into mice produced sedation, palpebral ptosis and analgesia. The injection of these peptides inhibited spontaneous rearing activity and prolonged hexobarbital-induced sleeping time (ZETLER, 1980b). Accordingly, CCK-8 is assumed to be antagonistic to the central action of TRH. In fact, the present study demonstrated that the duration of pentobarbital- and ethanol-induced narcosis was prolonged by CCK-8 and shortened by TRH, and that these effects were reduced when these two peptides were administered simultaneously.

COSTALL *et al.* (1979) reported that intraventricular or intracerebral injection of TRH into rats induced a variety of motor effects, such as shakes, limb tremor, repetitive head and limb movements, biting, scratching, and an alertness. Ac-

cording to CARINO *et al.* (1976), behavioral excitation was most pronounced after administration of TRH into the cerebral cortex, caudate nucleus, and hypothalamus. However, MASSERANO and KING (1981) have shown that locomotor activity increases after injection of TRH into the hypothalamus, but not into the caudate nucleus and septum. TRH receptor binding is likely to vary markedly in the brain regions. OGAWA *et al.* (1981) reported that the limbic system contained the greatest amount of binding sites, the next highest areas being the cerebral cortex, hypothalamus, interpeduncular nucleus, and periaqueductal gray matter of the midbrain. Thus, the sites of action of TRH are not simply determined.

Regarding the mode of action of TRH, an increase in the brain catecholamine turnover has been reported by a number of investigators, and the presynaptic dopamine release was suggested to be stimulated by TRH (REIGLE *et al.*, 1974; HORST and SPIRT, 1974; KERWIN and PYCOCK, 1979). GREEN *et al.* (1976) assumed that TRH modulates dopamine-mediated behavioral responses by a presynaptic mechanism of action, possibly by releasing dopamine. Furthermore, it was inferred that, of the two major dopamine terminal areas in the rat brain, the mesolimbic nucleus accumbens rather than the extrapyramidal nucleus caudatus is the site where TRH selectively modulates presynaptic dopaminergic mechanisms (HEAL and GREEN, 1979). In marked contrast to TRH, CCK-8 is likely to inhibit the release of dopamine in the presynaptic nerve endings (FUXE *et al.*, 1980). In this line of literature, it would be presumed that the antagonistic action of CCK-8 on TRH-induced excitation may be due to its inhibitory effect on dopamine release from the presynaptic nerve endings, particularly in the mesolimbic nucleus.

However, there might be different sites of action and mechanisms for CCK-8 and TRH. Thus, their effects may not always oppose each other. CCK-binding sites in the rat brain are correlated to its cerebral distribution (SAITO *et al.*, 1980) and they are not in accord with those for TRH. Accordingly, some effects of TRH, such as body shaking, are not antagonized by CCK-8. WEI (1981) demonstrated that injection of TRH into medial brainstem areas produced vigorous shaking, while injection into the lateral regions of the brain was relatively inactive. The body shaking-inducing areas are different from the brain regions containing massive amounts of CCK.

Stimulant properties of amphetamine are similar to those of TRH in various respects (MANBERG *et al.*, 1979). In the present experiments, hyperactivity induced by methamphetamine was effectively suppressed by CCK-8, as seen in the case of TRH. The observation apparently indicates a sedative action of CCK-8. One of the interesting findings in this study is that caerulein is ineffective in suppressing methamphetamine-induced hyperactivity. Moreover, CCK-8-NS itself produced an enhanced locomotor activity and it caused an enhancement of hyperactivity induced by methamphetamine when injected in relatively large

amounts. Since commercial caerulein preparations do not correspond to the label claim (DE CASTIGLIONE and DI SALLE, 1980), reasonable conclusions could not be drawn as to the effect of caerulein. However, the fact that CCK-8 had a sedative action, while CCK-8-NS enhanced locomotor activity suggests that the presence of the sulfate group on the tyrosine residue is indispensable for the exertion of the action of CCK in the inhibition of presynaptic dopamine release.

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