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Rotational Syndrome After Central Injection of C-Terminal 7-Peptide of Cholecystokinin

J. F. E. MANN, R. BOUCHER[1] AND P. W. SCHILLER[2]

Clinical Research Institute of Montreal, Montreal, Quebec

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MANN, J. F. E., R. BOUCHER AND P. W. SCHILLER. *Rotational syndrome after central injection of C-terminal 7-peptide of cholecystokinin.* PHARMAC. BIOCHEM. BEHAV. 13(1) 125–127, 1980.—Intracerebroventricular (ICV) injections of sulfated C-terminal 7-peptide of cholecystokinin and of its N-*tert*-butyloxycarbonyl-protected analog elicited rotational behavior (barrel rotations), accompanied by a distorted head and body position, lack of spontaneous motor activity other than rotations, characteristic limb flexion and extension and loss of some reflexes. When the unsulfated 7-peptide was ICV injected, none of the above symptoms was observed. The rotational syndrome described resembles the syndrome of acute unilateral labyrinthectomy. Since similar results have been reported following central injections of other peptides, however, it appears to be a non-specific, probably toxic effect.

Cholecystokinin Barrel rotations Intracerebroventricular injections Rats Labyrinthectomy Toxicity

CHOLECYSTOKININ was first isolated from the gut as a linear 33-peptide containing a sulfated tyrosyl residue in position 27 (CCK-33-S; S=O-sulfate) [15]. Recently, the presence of peptides resembling CCK-33-S and its C-terminal 8-peptide (CCK-8-S=H·Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe·NH_2) in the brain of several species [5, 14, 23], including man [18], was demonstrated by immunochemical methods. Subsequently, a peptide with the chemical structure of the C-terminal 8-peptide (CCK-8-S) was isolated from sheep brain [6]. The latter study revealed the existence of several other peptides related to the C-terminal sequence of the 33-peptide, whose chemical structures have not yet been established. It is, therefore, quite possible that the sulfated C-terminal 7-peptide of CCK (CCK-7-S) is also present in the brain. Both CCK-7-S and CCK-8-S are more active than CCK-33-S in gastrointestinal assay systems [1,7]. It remains to be elucidated which of the CCK-peptides is physiologically active in the brain.

Immunohistological and immunochemical evidence suggests that peptides resembling CCK-33-S and CCK-8-S are widely distributed in the brain and cerebrospinal fluid [14, 17, 18, 22]. The latter studies, however, did not rule the likely possibility that the antibodies used might cross-react with CCK-7-S. Therefore, the exact chemical structure of the immunoreactive material remains to be established. Cell bodies and nerve fibers containing CCK-like peptides were observed in the cerebral cortex, in the hypothalamus and in the hippocampus [8, 11, 14, 18, 22]. Immunoreactivity was also found in the periaqueductal gray [8] and in the medulla oblongata [11], although the latter finding could not be confirmed with another antibody preparation [8].

Little is known about the role of CCK-peptides in the central nervous system. *In vivo* CCK-8-S has been shown to inhibit feeding by peripheral and by intracerebroventricular (ICV) administration [4,16], and *in vitro* CCK-8-S releases growth hormone from the pituitary [13]. The unsulfated C-terminal 7-peptide (CCK-7) has recently been demonstrated to display opiate activity in the rat brain membrane receptor assay and in the guinea pig ileum assay [20]. While investigating the effects of CCK-peptides on blood pressure (unpublished results), we observed a rotational syndrome following ICV injection. The peptides studied included CCK-7, CCK-7-S and CCK-7-S with N-terminal protection by the t-butyl-oxycarbonyl (Boc) group (Boc-CCK-7-S).

METHOD

Male Wistar rats (Canadian Breeding Farm) (n=43) weighing 300–400 g were housed individually at constant temperature (24±2°C) and a 12 hour light/dark circle was maintained. The rats were implanted with 23 ga chronic cerebral cannulas into the right lateral cerebral ventricle as has been described in detail [12]. Coordinates with respect to bregma (flat skull) were 0.5 mm posterior, 1.3 mm lateral, and 5.5 mm deep from the skull surface. Patency of the cannula was tested 2 days after implantation by ICV injection of 100 ng angiotensin II. Only rats which started to drink within 2 min of angiotensin II administration were included in the study (for details see ref. [12]). Five to 8 days after ICV cannulation, a 30 ga injector was inserted into the chronic cannula. The injector was connected to a 50 μl Hamilton-syringe and test substances were injected in a volume of 10 μl over a period of 60 seconds. Thereafter, the rats were observed on a table (1×2 m) and reflexes (righting, contact

[1]Deceased on January 20, 1980.

[2]Reprint requests should be addressed to Dr. Peter W. Schiller, Director, Lab of Chem Biology and Peptide Research, Clinical Research Institute, 110 Pine Avenue West, Montreal, Que., Canada H2W 1R7.

TABLE 1
EFFECTS OF ICV APPLIED CCK-7 AND ANALOGUES

Effects	Number of rats showing symptoms out of the total tested			
	CCK-7	CCK-7-S	Boc-CCK-7-S	Ethanol/saline mixtures
Rotations	0/9	7/8	5/9	0/17
Lateral head/body position	0/9	7/8	5/9	0/17
Limb flexion/extension	0/9	7/8	5/9	0/17
Eye deviation	0/9	7/8	5/9	0/17
Loss of reflexes				
a) righting	0/9	4/8	2/9	0/17
b) contact placing	0/9	4/8	4/9	0/17
c) reaching	0/9	6/8	5/9	0/17

placing, reaching) [9], and reactions to tactile stimulation (tail lifting, slight touching) were evaluated after 5, 15, 30 and 45 min. A stop-watch was used to record frequency of rotations.

CCK-peptides were synthesized [1] and kindly donated by Professor Miklos Bodanszky, Dept. of Chemistry, Case Western Reserve University, Cleveland. Due to their lipophilic properties it was necessary to dissolve them in ethanol (EtOH)/0.9% NaCl (saline) mixtures. In the behavioral tests, peptides were administered to drug-naive animals as follows: (1) CCK-7 (n=9), 3×10^{-8} moles, dissolved in 40% EtOH/60% saline; (2) CCK-7-S (n=8), 4×10^{-8} moles, dissolved in 30% EtOH/70% saline; (3) Boc-CCK-7-S (n=9) 3×10^{-8} moles, dissolved in 50% EtOH/50% saline. In control experiments saline solutions containing 30% (n=5), 40% (n=4) and 50% (n=8) EtOH were injected. All rats were examined using one test compound only.

RESULTS

When CCK-7-S was applied ICV all but one rat tested (Table 1) characteristically placed one side of the head (right in 6, left in 1) on the surface of the table within 2 min. Some rats turned the whole body on one side. The ipsilateral limbs were flexed and the contralateral ones extended. The contralateral eye showed an upward deviation. Another 1 to 3 min later the rats started to rotate along the longitudinal body axis ("barrel rotations") either spontaneously or in response to tail lifting or slight touching. The direction of rotations was the same as that initially observed for the lateral head position. The interference of a vertical wall slowed down the rate of rotations, but failed to prevent them. The rate of rolling was 2–5/sec and spontaneous rotations occurred in "bursts", one burst lasting for 10–20 sec. Initially, fits of barrel rotations took place about 3–4 times/min, becoming less frequent later on. No rotational activity was observed after 15 min. Between rotational fits and 10–15 min after their cessation the rats resumed the initial distorted posture. When rats were manipulated into another position, they immediately resumed their initial posture or started to rotate. Nystagmus was also observed. There was, however, no spontaneous locomotor activity other than barrel rolling. Reflexes could not be reliably evaluated during the first 15 min, since handling provoked barrel rotations. When the symptoms described above vanished, reflexes could not be elicited in some of the animals (Table 1). At that time, spontaneous locomotor activity was still absent. No pathological signs and no prostration were observed 45 min after CCK-7-S injection.

In 5 out of 9 Boc-CCK-7-S treated animals we found the same symptoms as in rats injected with CCK-7-S (Table 1). However, 2 of the rats died of respiratory arrest 8 min after ICV injection and after about 5 min of intense barrel rolling. With the other 3 animals barrel rotations were observed for 25–30 min. All 5 rats rotated leftward and the initial placement of the head and turning of the body was also to the left side. After cessation of the rotational syndrome, contact placing and reaching reflexes could not be elicited in the 3 surviving rats, and the righting reflex in 2 of them. The former 2 reflexes were also absent in other rats, who showed no rotational behavior (Table 1). No pathological signs were detected 60 min after Boc-CCK-7-S injection.

Unsulfated CCK-7 and EtOH/saline mixtures induced none of the symptoms described for the sulfated analogues at the same dose (Table 1).

DISCUSSION

Sulfated CCK-7-S peptides provoke an unusual rotational behavior along the longitudinal body axis. These barrel rotations have also been noticed after ICV injection of somatostatin (ST) [3] and substance P (SP) [2] in the same dose range as with CCK-7-S, and of vasopressin (VP) and VP-analogues [10] at lower doses. ST elicited barrel rolling of a duration comparable to that of CCK-7-S and in contrast to the much shorter effects of SP and VP. Sedation and prostration were observed in ST- and VP-treated animals for several hours. Rats receiving CCK-7-S appeared rather normal after 30–40 min. From the description of barrel rotations following SP, ST and VP [2, 3, 10] and our own observation it appears, that central injections of peptides may induce a common syndrome. It is thus conceivable that we are dealing with an unspecific effect, probably a toxic effect, since 2 rats even died after testing with Boc-CCK-7-S.

CCK-7-S applied to the brain evokes a series of symptoms or syndrome which has a resemblance to the effects of acute unilateral labyrinthectomy [19]. Destruction of one of the two organs leads to rotations, which are thought to depend on the unopposed action of the intact labyrinth. Furthermore, the eyes are deviated, the head is turned to the side of the lesion, the limbs ipsilateral to the lesion are flexed and the contralateral ones are extended [19]. Nystagmus is also present. Humans with acute loss of one labyrinth remain in the least uncomfortable position avoiding any positional

changes, which inevitably would increase the vertigo. Apparently, CCK-7-S evokes a similar syndrome. However, we cannot explain why the presumably toxic peptide action on the central nervous system results in such a complex behavior. The resemblance to unilateral labyrinthectomy may be a coincidence. In some of the rats a loss of reflexes and a reduced locomotor activity was observed. This might be a consequence of the equilibrium disturbance or an effect of CCK-7-S on its own.

CCK-7 did not elicit a rotational syndrome at the dose used. On the other hand, pin-prick and tail-flick tests revealed a naloxone-reversible analgesic activity of this compound in 2 out of 9 rats (unpublished results). The analgesic effect is compatible with the recently demonstrated [20] *in vitro* opiate activity of CCK-7 which can be explained on the basis of structural similarities between the latter peptide and methionine-enkephalin [21]. While a sulfated tyrosyl residue is detrimental to analgesic activity, the present data show it to be a crucial structural element for eliciting barrel rolling. The latter observation could be due to a direct structure-toxicity effect. Alternatively, CCK-7 might be more efficiently degraded by enzymes or taken up by cells than CCK-7-S and Boc-CCK-7-S. These findings may be of interest for further investigations on the role of CCK-related peptides in the brain, because the doses we have employed were only about 3–5 times higher than the threshold dose of CCK-8 ICV reported to inhibit feeding in rats acutely [16].

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