EFFECT OF C-TERMINAL CHOLECYSTOKININ TETRAPEPTIDE (CCK-4) ON FUNCTION OF THE ISLETS OF LANGERHANS AND ADENOHYPOPHYSIS

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The C-terminal tetrapeptide of cholecystokinin, containing the amino-acid sequence H-Trp-Met-Asp-Phe-NH_2 (Trimafam, CCK-4), is also a structural fragment of gastrin and cerulein. It has been shown [9] that CCK-4, together with cholecystokinin, is present in nerve endings in the pancreatic islets of Langerhans. After administration to man, and also during perfusion of the pig pancreas, the tetrapeptide can stimulate the secretion of pancreatic hormones [7, 9].

The object of this investigation was to study the direct action of C-terminal cholecystokinin tetrapeptide on the secretory function of the A-, B-, and D-cells of the islets of Langerhans of the rat pancreas. Considering the neurotransmitter role of cholecystokinin in the CNS, the possible direct action of CCK-4 on the adenohypophysis and, in particular, on its lactotrophic function, also was investigated.

EXPERIMENTAL METHOD

CCK-4 was obtained by chemical synthesis, by a scheme embodying stepwise addition of amino-acid residues in solution, and based on the use of amides of a C-terminal amino acid (L-phenylalanine) and intermediate peptides as the amino components, and pentafluorophenyl esters of correspondingly protected amino acids as the activated carboxyl components. After synthesis of the partially protected tetrapeptide, it was demasked and purified to obtain CCK-4 (monohydrate-monohydrochloride) in an analytically pure state. The preparation of CCK-4 used in the subsequent experiments possessed the following constants: m.p. 162–163°C (decomposition); $[\alpha]_D$ 25–31.5° (C 1.0; dimethylformamide); R_f 0.70 (n-butanol–acetic acid–water, 4 : 1 : 1; TLC on Silufol UV-254 plates). The experiments in vivo were carried out on male Wistar rats weighing 200 g. CCK-4 in physiological saline was injected into the jugular vein under superficial ether anesthesia. The animals were killed by decapitation 2 and 15 min after injection. Blood serum was kept at –20°C. Experiments in vitro were carried out on a 4-day culture of islet cells of newborn rats or on 4-day monolayer cultures of adult rat adenohypophysis. The technique of preparation and management of the cultures was described previously [2–4]. Cell cultures were grown in medium No. 199 with the addition of 10% embryonic calf serum. The tetrapeptide for investigation was added to the culture medium and incubated with cells of islets of Langerhans for 30 min and with adenohypophysis cells for 2 h. After incubation the medium was separated from the cells, frozen, and kept at –20°C. Hormones in the medium and insulin in the blood serum were determined by radioimmunoassay using kits from RSL, USA (glucagon), INC, USA (somatostatin), and the Isotopes Institute, Hungary (insulin). The prolactin concentration in the medium was determined by radioimmunoassay, by a method developed in the writers' laboratory, using prolactin isolated from rats and purified (for iodination and as the standard), and also a highly specific antiserum against it [1]. A gamma-counter (Searle) was used for the radiometric analysis.

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TABLE 1. Effect of Intravenous Injection of Tetrapeptide on Blood Insulin Level (μU/ml) in Rats

Expt. No.	Dose of preparation	Time after injection of preparation, min	
		2	15
1	Physiological saline	27,1±1,5 (7)	
	5 μg/kg	40,8±3,0 (16) $P<0,001$	
2	Physiological saline		16,6±1,6 (8)
	5 μg/kg		25,8±2,2 (10) $P<0,01$
	50 μg/kg		29,8±3,5 (9) $P<0,01$

Legend. Number of animals given in parentheses.

TABLE 2. Effect of Tetrapeptide on Secretion of Insulin, Glucagon, and Somatostatin by Culture of Rat Islet Cells

Dose of preparation, M	Concentration in medium		
	insulin, μU/ml	Glucagon, pg/ml	somatostatin, pg/ml
0	333,5±21,2 (7)	533,5±104,3 (4)	2,91±0,55 (10)
10^{-10}	298,0±19,9 (8)	531,0±41,0 (8)	5,75±0,80 (12)
10^{-9}	549,0±7,4* (7)	810,0±68,9* (8)	11,43±0,90* (9)
10^{-8}	585,4±92,0* (7)	3800±649* (5)	12,74±1,10* (10)
10^{-7}	667,0±58,4* (8)	4076±502* (8)	12,50±1,50* (12)
10^{-6}	796,0±80,0* (6)	4047±356* (8)	15,44±1,85* (12)
10^{-5}	741,0±66,0* (7)	4910±866* (8)	15,65±1,38* (14)

Legend. Number of observations in parentheses. *$P < 0.05$.

EXPERIMENTAL RESULTS

Intravenous injection of CCK–4 into rats in doses of 5 and 50 μg/kg body weight led to a significant increase in the blood immunoreactive insulin concentration. This effect was found as early as 2 min after injection of the preparation and it continued for at least 15 min (Table 1). Having confirmed that the preparation is highly effective as a stimulator of insulin secretion, as Rehfeld et al. [7] discovered previously on volunteers, tests were carried out on rat pancreatic islet cells in culture in order to obtain proof of the direct action of CCK–4 on secretory activity of the hormone-secreting cells.

The results of experiments showing the effect of the tetrapeptide on function of three types of cells of the islets of Langerhans, secreting insulin (B-cells), glucagon (A-cells), and somatostatin (D-cells), are given in Table 2. By using a wide range of doses of CCK–4 it was possible to show that, starting with a dose of 10^{-9} M, the tetrapeptide had a statistically significant stimulating action on secretion of all three hormones studied. Within the dose range from 10^{-9} to 10^{-6} M, the dose–effect relationship was linear in character.

Considering that cholecystokinin and its fragments are widely represented in the brain and peripheral nervous system [5, 6], an attempt was made to discover whether CCK–4 has a direct action also on function of the adenohypophysis, in view of the possible transport of this peptide fragment from the hypothalamus into the anterior lobe of the pituitary through the system of its portal vein.

The results in Table 3 show conclusively that CCK–4 has no direct stimulating action on secretory activity of the adenohypophysis or, at least, on its lactotrophic function. In doses causing intensive stimulation of hormone secretion by the islets of Langerhans, in the course of incubation for 2 h the tetrapeptide had no effect on either basal or dopamine-inhibited prolactin secretion by adenohypophyseal cells in culture.

The investigations of Rehfeld et al. [8, 9] showed that the intestinal hormones gastrin and cholecystokinin are synthesized in nerve cells and accumulate in synaptic vesicles. The use of an immunohistochemical technique revealed the presence of the C-terminal cholecystokinin tetrapeptide in nerve endings of the islets of Langerhans. Perfusion of pig pancreas with the tetrapeptide led to an increase in the concentration of pancreatic hormones in the perfusion fluid; the effect of the C-terminal fragment, moreover, was more marked than the effect of cholecystokinin itself [9].

TABLE 3. Effect of Tetrapeptide on Basal and Dopamine-Inhibited Secretion of Prolactin by Rat Adenohypophyseal Cell Culture

Expt. No.	Group	No. of observations	Prolactin concentration in medium, ng/ml ($M \pm m$)	P
1	Control	4	3393±717	
2	Tetrapeptide (10^{-6} M)	5	2938±285	1—2>0,05
3	Tetrapeptide ($5 \cdot 10^{-6}$ M)	3	2419±109	1—3>0,05
4	Dopamine (80 ng)	4	602±39	1—4<0,01
5	Dopamine (80 ng) + tetrapeptide (10^{-6} M)	5	614±59	4—5>0,05
6	Dopamine (80 ng) + tetrapeptide ($5 \cdot 10^{-6}$ M)	4	663±122	4—6>0,05

Consequently, the C-terminal tetrapeptide of cholecystokinin stimulates the secretion of insulin, glucagon, and somatostatin, through direct contact between the peptide and target cells. The rapid response of islet cells to the tetrapeptide present in the culture medium suggests that specific receptors are present on their membranes. On the other hand, the absence of response of the lactotrophs suggests that CCK-4 does not bind specifically with the hormone-secreting cells of the adenohypophysis, and, hence, that CCK-4 has no physiological role in the regulation of pituitary functions.

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