Effect of a Cholecystokinin Preparation on Brain Monoamines in the Rat

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Summary In order to elucidate the central action mechanism of cholecystokinin (CCK), effects of this peptide on norepinephrine, dopamine and serotonin contents in the brain were observed in saline control, α -methyl-*p*-tyrosine-, L-DOPA- and pargyline-pretreated rats. Intraperitoneal injection of CCK in a dose of 40 U/kg did not produce any significant changes in the monoamine contents. However, since a tendency to increase in norepinephrine turnover rate after CCK injection was noticed, the possibility that brain monoamines are involved in the central action of CCK could not be excluded.

Our previous study indicated that intraperitoneal as well as intraventricular injection of a cholecystokinin (CCK) preparation caused a pronounced enhancement of pituitary adrenocortical secretion (ITO *et al.*, 1979). Effect of C-terminal octapeptide of CCK on the secretion of prolactin, growth hormone, luteinizing hormone and thyrotropin were also reported by VIJAYAN *et al.* (1979) and MORLEY *et al.* (1979). The role of hypothalamic monoamines in the secretion of these pituitary hormones has been extensively studied by many investigators. Moreover, CCK is known as a putative satiety hormone which regulates food intake (*cf.* MUELLER and HSIAO, 1978). Regulation of feeding behavior by hypothalamic monoamines was shown by a number of researchers (GROSSMAN, 1962; DAVIS and KEESEY, 1971). Accordingly, it might be assumed that the central effect of CCK is mediated by changes in the brain monoamine metabolism. From this point of view, the present study was carried out to observe the effect of CCK on the brain monoamine contents in rats treated with α -methyl-*p*-tyrosine (α -MT), L-DOPA and pargyline.

Male Wistar rats, weighing approximately 250 g, were used throughout the experiments. They were housed at a constant temperature of $25 \pm 2^\circ\text{C}$ under a light: dark ratio of 12: 12 hr with lights turned on at 07:00. Rat biscuits (Oriental Yeast Co.) and drinking water were available *ad libitum*. CCK used in the present experiment was a commercial preparation manufactured by Boots

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Co. and its potency was expressed in terms of Harper unit. Other drugs used were α -MT (Sigma Co.), L-DOPA (Nakarai Co.) and pargyline (Nakarai Co.). Rats were injected i.p. with α -MT in a dose of 200 mg/kg body weight 12 hr before, injected i.p. with L-DOPA in a dose of 100 mg/kg 1 hr before, or given pargyline *per os* in a dose of 40 mg/kg 2 hr before the experiments. These pretreated animals were injected i.p. with CCK in a dose of 40 U/kg which has been shown to induce a marked increase in the plasma corticosterone level (ITO *et al.*, 1979) or isotonic saline in the morning at 09:00, and 30 min later they were sacrificed by decapitation.

The brain was removed as quickly as possible, placed on ice and divided into the telencephalon, diencephalon, hypothalamus and pons+medulla oblongata according to GLOWINSKI and IVERSEN (1966). Hypothalamic tissue obtained from 3 rats was pooled and all samples were kept frozen until analysis. The samples were homogenized in cold 0.4 N perchloric acid, centrifuged for 10 min at $1,000 \times g$, and their supernatants were analysed for norepinephrine, dopamine and serotonin contents by the method of KARASAWA *et al.* (1975). Briefly the supernatant was first passed through double columns consisting of an aluminum oxide column (0.6×1.0 cm) and an Amberlite CG-50 column (0.6×1.5 cm). Norepinephrine and dopamine were adsorbed on aluminum oxide in the upper column, while serotonin was retained by Amberlite CG-50 in the bottom column. After washing the columns with distilled water, the amines were eluted with diluted hydrochloric acid. Catecholamines in the eluate were then transformed to fluorescent trihydroxyindole compounds by an oxidation method. As the oxidant, iodine and ferricyanide were used for norepinephrine and for dopamine, respectively. Fluorescence development of serotonin was performed by *o*-phthalaldehyde conjugation. Fluorescent intensities of these samples were measured on a fluorescence spectrometer (Hitachi MPF-2A). For statistical analysis of the data Student's *t*-test was used.

Results are summarized in Table 1. In control rats the norepinephrine content was particularly high in the hypothalamus as compared with the values in other parts of the brain, and the highest content of dopamine was seen in the telencephalon. Serotonin was distributed evenly in the brain, although the content was slightly less in the telencephalon. α -MT, which inhibits tyrosine hydroxylase, caused marked decreases in the dopamine and norepinephrine contents, while L-DOPA, precursor of dopamine, produced a large increase in the dopamine content in all regions of the brain. Pretreatment with pargyline, MAO inhibitor, also caused a significant increase of monoamines. However, when the contents of these monoamines after CCK injection were compared with the respective values of saline controls, no difference was observed, except in one case where the dopamine content in pons+medulla oblongata showed a greater decrease after CCK injection in the α -MT pretreated group.

Although changes were not significant, a tendency to decrease in the norepinephrine content after CCK injection may not be overlooked; that is, the

Table 1. Effect of cholecystokinin (CCK) preparation (40 U/kg, i.p.) on brain monoamine contents in rats treated with α -methyl-*p*-tyrosine (α -MT), L-DOPA and pargyline.

Region group	Treatment	No. of rats	Norepinephrine	Dopamine	Serotonin
			ng/g wet weight (mean±SEM)		
Telencephalon					
Control	Saline	10	347±11.7	1224±39.0	414±24.3
	CCK	10	343± 7.2	1256±50.2	430±23.7
α-MT	Saline	18	142±17.6 ^a	547±81.6 ^a	335±18.4 ^c
	CCK	18	115±16.9 ^a	413±70.6 ^a	343±16.1 ^b
L-DOPA	Saline	18	327±10.2	2050±132.2 ^a	347±17.2 ^c
	CCK	18	312± 8.1 ^c	2036±120.8 ^a	354±12.9 ^b
Pargyline	Saline	9	474±19.4 ^a	1658±57.4 ^a	516±18.1 ^b
	CCK	10	445±10.4 ^a	1515±46.3 ^b	523±20.9 ^b
Diencephalon					
Control	Saline	15	490±10.8	150± 6.0	667±49.5
	CCK	15	499±14.7	156±13.6	677±58.0
α-MT	Saline	18	237±27.9 ^a	75±10.2 ^a	454±39.0 ^b
	CCK	18	188±22.3 ^a	76±13.0 ^a	508±52.4 ^c
L-DOPA	Saline	18	560±21.6 ^c	912±116.2 ^a	599±33.4
	CCK	18	541±16.5	780±84.7 ^a	647±46.7
Pargyline	Saline	18	612±14.4 ^a	239±19.3 ^a	1001±51.3 ^a
	CCK	18	591±11.9 ^a	238±21.3 ^b	1042±45.4 ^a
Hypothalamus					
Control	Saline	18 (6)*	1303±90.7	326±25.7	605±88.6
	CCK	18 (6)	1202±54.8	313±19.3	789±137.8
α-MT	Saline	18 (6)	626±88.1 ^a	96±19.3 ^a	411±94.0
	CCK	18 (6)	486±42.6 ^a	88±17.7 ^a	447±127.7
L-DOPA	Saline	18 (6)	1124±69.2	1032±69.6 ^a	464±90.1
	CCK	18 (6)	1128±56.2	1007±137.3 ^a	552±109.9
Pargyline	Saline	18 (6)	1455±71.7	427±36.1 ^c	943±91.7 ^c
	CCK	18 (6)	1449±49.2 ^b	402±27.4 ^c	1011±56.0 ^a
Pons + Medulla oblongata					
Control	Saline	10	583±29.6	75±14.3	663±29.2
	CCK	10	586±20.5	87±14.3	701±54.7
α-MT	Saline	18	258±26.5 ^a	51± 5.7	632±34.7
	CCK	18	195±22.1 ^a	32± 4.0 ^{c, d}	607±44.6
L-DOPA	Saline	18	554±10.6	835±108.3 ^a	598±30.2
	CCK	18	568±12.3	769±97.8 ^a	614±25.7
Pargyline	Saline	9	734±20.2 ^a	97±14.5	860±33.5 ^a
	CCK	10	701±44.3 ^c	109±13.4	890±31.4 ^b

^a, $P < 0.001$; ^b, $P < 0.01$; ^c, $P < 0.05$ vs. respective values of control group in the same region; ^d, $P < 0.01$ vs. saline level in the same subgroup. * Six pooled samples obtained from 18 rats.

average decreases were 19% in telencephalon, 21% in diencephalon, 22% in hypothalamus and 24% in pons+medulla oblongata. The results are suggestive of the participation of norepinephrine in the central action of CCK, but direct evidence for an increased turnover rate of norepinephrine is required. The action mode of CCK other than controlling the monoamine metabolism should be investigated in further studies. The important role of monoamines in various central nervous functions are known, and the central action of CCK has attracted considerable attention by many investigators because of its abundance in the brain (DOCKRAY, 1976; REHFELD, 1978; REHFELD *et al.*, 1979). The present observations make up one step of a series of experiments on the central action of CCK.

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