Ratio between large and small carboxy-terminal molecular forms of cholecystokinin in brains of different species[1]

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Summary. The ratio between large and small carboxy-terminal forms of cholecystokinin in brain extracts from man, pig, dog, rat, chicken, frog and trout was determined by two sequence-specific radioimmunoassays. It was found that the relative amounts of large forms of cholecystokinin; are higher in mammalian brain than in brains of lower species.
Key words. Cholecystokinin; radioimmunoassay; species difference; molecular heterogeneity; brain.

Cholecystokinin (CCK) is one of the regulatory peptides found both in intestine and brain of different species, including mammals, birds, amphibians, and fishes[2-15]. It has been shown that CCK in the brain is heterogeneous[2,7,9,10-15]. The most abundant molecular form of CCK in brain co-elutes with synthetic sulfated CCK-8. In addition to this small CCK form, some workers have demonstrated the presence of large molecular forms of CCK in brain extracts[10,14,15], whereas others, using similar fractionation and radioimmunoassay (RIA) techniques, were unable to detect these large forms of CCK[3,4,8].

We have developed a RIA-system specific for large carboxy-terminal forms of CCK[16-18]. Using this assay we have demonstrated that large carboxy-terminal forms of CCK do exist in the brain. By comparing the results of this assay with those obtained by a RIA system using an antibody that predominantly binds to small carboxy-terminal forms of CCK, we have determined the ratio between large and small forms of CCK in aqueous and acid extracts of brains from several species.

Methods. Brain tissue was obtained about 8 h after death in humans, and immediately after death in the other species. Specimens of cerebral cortex of man, dog, pig, rat and chicken, and whole brains of frog *(Rana esculenta)* and fish *(Salmo irideus)* were divided into equal parts and immediately extracted either in boiling water (1 g/10 ml) or in 0.5 M boiling acetic acid (1 g/10 ml) for 10 min. After homogenization in a pyrex tissue grinder (Kontes Glass Co. Vineland, NJ, USA) and centrifugation (5000 × g for 10 min), the supernatant was rapidly frozen, lyophilized and redissolved in assay buffer prior to assay. All tissue extracts were measured by RIA using two antibodies with different specificities. Antibody 1703 is specific for large carboxy-terminal molecular forms of CCK[17,18], whereas antibody 5135 binds predominantly to small carboxy-terminal molecular forms of CCK. For the purpose of this study a bleed of antibody 5135 with low binding to large forms of CCK was selected (table 1). Both antibodies were raised in rabbits, antibody 1703 against 30% purified porcine CCK-33, and antibody 5135 against synthetic non-sulfated CCK-8 coupled to bovine serum albumin. In the assay using antibody 1703, 99% pure porcine CCK-33 (V. Mutt, Karolinska Institute, Stockholm, Sweden) was used as standard, and 99% pure porcine CCK-33 coupled to ^{125}I hydroxyphenylpropionic acid succinimide ester was employed as label[10,16-18], whereas in the assay using antibody 5135, sulfated CCK-8 was used as standard preparation, and non-sulfated CCK-8 (Squibb & Sons, Princetown, NJ, USA) coupled to ^{125}I by the chloramine-T method was used as label[18]. 0.05 M sodium phosphate pH 7.4 containing 0.08 mmol/l human serum albumin and 0.06 mmol/l sodiumethylmercurithiosalicylate was employed as assay buffer. Separation between free and antibody bound label was performed by adsorption of the free peptide to a charcoal suspension. The 50% inhibition dose (ID_{50}) was 2.8 pmol/l for antibody 1703 and 3.5 pmol/l for antibody 5135. The intra-assay coefficient of variation for the antibodies ranged from 4.6 to 11.6% and the interassay coefficient of variation ranged from 11.3 to 26.1%. The concentration of CCK-like immunoreactivity was expressed as pmol per gram wet weight of tissue (pmol/g).

Results were expressed as the mean ± standard error of the mean. Statistical analysis was performed using Student's t-test.

Results and discussion. Table 2 shows the concentrations of carboxy-terminal CCK in aqueous and acid extracts of brains from different species as measured by RIA using antibody 1703 (large CCK) and antibody 5135 (small CCK), respectively. The ratio between large and small forms of CCK in both aqueous and acid extracts of mammalian brain ($n = 12$) was significantly ($p < 0.001$) higher than in the brains of the other species studied ($n = 10$). Fractionation of the aqueous-acid extract of porcine brain on a Sephadex G50 SF column measured by RIA using antibody 1703 revealed multiple carboxy-terminal molecular forms of CCK larger than sulfated CCK-8, whereas RIA using antibody 5135 showed one predominant molecular form eluting at the position of synthetic sulfated CCK-8 (fig.).

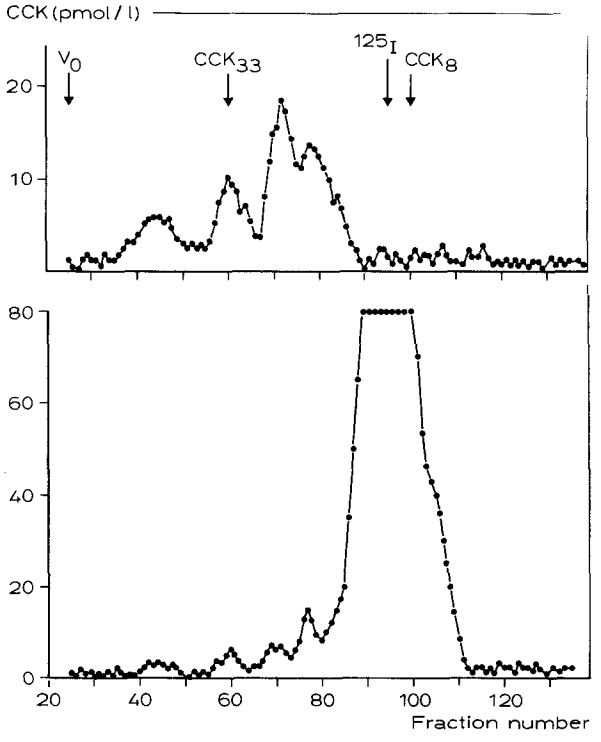
The main findings of the present study can be summarized as follows; 1) large carboxy-terminal CCK-peptides are present in

Table 1. Binding of different cholecystokinin (CCK)-peptides to antibody 5135 and antibody 1703

	Ab 5135	Ab 1703
CCK 39	0.20	0.90
CCK33	0.20	1.00
Caerulein	0.90	< 0.01
CCK 8 sulfated	1.00	< 0.01
CCK 8 non-sulfated	1.00	< 0.01
CCK 4	< 0.01	< 0.01
NH_2-terminal CCK 15	< 0.01	< 0.01
NH_2-terminal CCK 21	< 0.01	< 0.01

Table 2. Concentrations of carboxy-terminal cholecystokinin-like immunoreactivity measured by radioimmunoassay using antibody 5135 and antibody 1703 in aqueous and acid extracts of brain from different species

Species	Aqueous extracts CCK-LI (pmol/g)			Acid extracts CCK-LI (pmol/g)		
	Ab 5135 (small CCK)	Ab 1703 (large CCK)	Large CCK-LI / Small CCK-LI	Ab 5135 (small CCK)	Ab 1703 (large CCK)	Large CCK-LI / Small CCK-LI
Man (n = 3)	107.2 ± 3.3	46.4 ± 2.7	0.43 ± 0.02	37.2 ± 1.6	80.7 ± 5.7	2.24 ± 0.21
Dog (n = 3)	90.2 ± 10.9	39.6 ± 3.7	0.45 ± 0.04	30.6 ± 5.7	67.6 ± 13.4	2.26 ± 0.37
Pig (n = 3)	78.7 ± 9.7	15.1 ± 2.0	0.19 ± 0.02	13.0 ± 1.7	41.3 ± 3.0	3.26 ± 0.19
Rat (n = 3)	70.4 ± 22.5	19.2 ± 3.8	0.30 ± 0.04	22.3 ± 9.8	44.2 ± 9.7	2.46 ± 0.55
Chicken (n = 3)	15.5 ± 1.4	< 1.0	< 0.05	10.5 ± 0.4	7.7 ± 0.2	0.74 ± 0.04
Frog (n = 4)	61.9 ± 4.5	4.7 ± 1.8	0.07 ± 0.03	44.7 ± 9.4	34.3 ± 4.7	0.65 ± 0.05
Trout (n = 3)	26.8 ± 10.3	< 1.0	< 0.05	11.0 ± 1.5	7.3 ± 1.5	0.67 ± 0.11



Fractionation of an aqueous-acid extract of porcine brain by Sephadex G50 column chromatography. The column was previously calibrated with bovine serum albumin (V_0), CCK33, sulfated CCK 8 and Na ^{125}I. The upper panel shows the results as measured by radioimmunoassay using antibody 1703 and the lower panel those obtained with antibody 5135.

the brain and 2) the ratio between large and small carboxy-terminal molecular forms of CCK is higher in mammalian brains than in the brains of non-mammalian species. This study further confirms previous reports showing that large basic molecular forms of CCK are preferentially extracted in acid, whereas small molecular forms of CCK are better extracted in boiling water (table 2)[3-6, 13, 14].

There is considerable controversy as to whether large forms of CCK are present in the brain. In some reports large forms of CCK have been demonstrated in mammalian brain[7, 13, 14], whereas other studies failed to detect such large molecular forms[3, 4, 8]. The inability of some workers to demonstrate large forms of CCK in mammalian brain might be attributable to several factors, such as the RIA system, the fractionation method or possibly the activation of a CCK converting enzyme[8, 14]. Studies on biosynthesis of CCK and molecular cloning of preprocholecystokinin support the finding that there are large molecular forms of CCK in the brain of dogs and rats[9, 15, 19].

The large form of CCK demonstrated in the present study differs from the large molecular form detected by Eng et al.[7]. Using a porcine-specific amino-terminal CCK-antibody, Eng et al. found that the large molecular form of CCK in porcine brain extracts represents amino-terminal desoctapeptide CCK, a CCK-fragment formed by the cleavage of CCK-8 from CCK-33. In contrast to the present report these authors were unable to demonstrate large carboxy-terminal forms of CCK[7]. The RIA-system used by Eng et al. differs considerably from the one used in this study, because their antibody was directed to the amino-terminus of CCK-33 and did not bind to CCK from other species apart from the pig[7]. Our RIA-system for large CCK (antibody 1703) is directed against the biologically active carboxy-terminal part of the CCK molecule and therefore this assay does not allow conclusions to be drawn about the presence of the amino-terminal desoctapeptide CCK. However, it is very well possible that both intact carboxy-terminal forms of CCK, as presented in this study, and the amino-terminal desoctapeptide CCK-fragment, as demonstrated by Eng et al.[7], are present in the brain.

In the absence of highly purified standard preparations of CCK from other species than the hog, it is impossible to determine the relative binding of antibodies to CCK of these species. However, using highly purified porcine CCK-33 and synthetic sulfated CCK-8 as standard preparations, we have shown that relative concentrations of large carboxy-terminal forms of CCK are higher in mammals than in lower species. Until now no other comparative studies on CCK forms in brains of various species have been performed. However, our findings are in agreement with results of two previous reports showing that the amounts of large forms of CCK in brains of lower species are very low[6, 20]. Furthermore, the presence of small amounts of large forms of CCK have been demonstrated in brain extracts of the frog[20].

In conclusion, the present study shows that large carboxy-terminal forms of CCK are present in the brain and that in the brains of different species the relative amounts of large and small carboxy-terminal molecular forms of CCK vary. In mammals the relative amount of large forms of CCK-like immunoreactivity is substantial, whereas in lower species the amount of large forms of CCK in the brain is negligible. Since the physiological role of CCK in the brain is unknown, the significance of the present finding awaits further studies.

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