Developmental and regional changes of cholecystokinin mRNA in rat brains

Makoto Hasegawa*, Hiroshi Usui, Kazuaki Araki, Ryozo Kuwano and Yasuo Takahashi

*Department of Psychiatry, School of Medicine and Department of Neuropharmacology, Brain Research Institute, Niigata University, Niigata 951, Japan

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Since the nucleotide sequence of cholecystokinin (CCK) cDNA was found in the rat gene, we applied cDNA to quantitate the CCK mRNA. The size of the mRNA for a CCK precursor was 850 nucleotides in length using brain cytoplasmic RNA. There were no bands except CCK mRNA by Northern blot analysis. We also examined the developmental changes and regional distribution of CCK mRNA in rat brains by dot-blot and gel-blot hybridization using CCK cDNA as a probe. CCK mRNA was barely detectable in the fetal brain, but started to increase postnatally and attained the plateau level after 20–30 days. Further, the level of CCK mRNA was highest in the frontal cortex, followed by those of the hippocampus and striatum. The cerebellum contained only negligible CCK mRNA. These results are in agreement with those of CCK concentration in the corresponding brain areas and suggest a transcriptional control of CCK concentration.

Cholecystokinin mRNA cDNA (Brain) Development Dot-blot hybridization

1. INTRODUCTION

Cholecystokinin (CCK) has long been recognized as a gut hormone. Recently several researchers reported that immunoreactive CCK-like peptides exist in the brain, particularly in neurons. Thus, it was suggested that CCK may act as a neurotransmitter or a neuromodulator [1–3]. Very recently several groups including ours succeeded in cloning the cDNA to mRNA of CCK [4–7]. We determined the nucleotide sequence of the inserted cDNA and deduced the amino acid sequence and proteolytic cleavage sites of the CCK precursor [4]. The availability of CCK cDNA probes now allows us to examine the levels of CCK mRNA in the brain and the structure of the CCK gene. Here we describe evidence for the presence of the CCK gene in the genomic DNA isolated from the rat brains, and examine the developmental and regional changes of CCK mRNA in rat brains using CCK cDNA as a probe.

2. MATERIALS AND METHODS

2.1. Animals

Wistar strain rats were from the animal room of our Brain Research Institute.

2.2. Southern blot hybridization analysis

Total high-molecular-mass genomic DNA was isolated from brains using the procedure of Blin and Stafford [8]. The DNA was digested with 2 restriction endonucleases (EcoRI and BamHI) and the DNA digests were electrophoresed on 0.5% agarose gel. The DNA fragments were transferred onto nitrocellulose membranes and hybridized with nick-translated 32P-CCK cDNA [9]. The [32P]cDNA probe for these hybridizations was prepared as follows: 10 μg plasmid DNA from a clone (pRC19) containing a sequence complementary to CCK mRNA [4] was digested with PstI, isolated by preparative acrylamide gel electrophoresis and labeled by nick-translation to a specific activity of about 10^8 cpm/μg.
2.3. RNA dot-blot hybridization and Northern blot hybridization analysis

Total cytoplasmic RNA was isolated from whole brains of developing rats by the phenol-chloroform-isoamyl alcohol extraction procedure [10]. Further, adult rat brains were dissected into frontal cortex, cerebellum, hippocampus and striatum. Total cytoplasmic RNA was isolated from the tissue of each brain area by the same procedure. Dot-blot and Northern blot hybridization analyses of mRNA were carried out essentially as described in [4,10].

3. RESULTS AND DISCUSSION

3.1. Evidence for CCK gene in total genomic DNA

Rat genomic DNA was analyzed by Southern blot analysis to characterize the CCK gene sequence. Nick-translated $^{32}$P-CCK cDNA hybridized to a single band or 2 bands per DNA digest: 2 bands of comparable intensity were found after BamHI digestion and a single band was found after EcoRI digestion (not shown).

These results show the existence of a sequence corresponding to CCK cDNA in total genomic DNA, indicating the presence of the CCK gene. The 2 bands observed in the Southern blot of the BamHI digest can be explained by the presence of an intron containing a restriction site for this endonuclease.

3.2. Changes of CCK mRNA in developmental rat brains

Northern transfer analysis of brain cytoplasmic RNA from the frontal cortex and cerebellum using CCK cDNA as a probe showed that the size of CCK mRNA was about 850 nucleotides in length (fig.1). This is in agreement with our previous data using brain microsomal poly(A) RNA. This figure indicates that there are no other bands except CCK mRNA; therefore the use of dot-blot analysis may be suitable for quantitation of CCK mRNA. Dot-blot analysis revealed that this CCK mRNA was barely detectable in fetal rat brains, but started to increase postnatally and attained a plateau level after 20–30 postnatal days (fig.2). Brandt [11] and Varro et al. [12] described the developmental increase of CCK-like activity in the brain using a radioimmunoassay. Our data on CCK mRNA cor-

Fig.1. Northern blot analysis of CCK mRNA. The cytoplasmic RNA from rat brains was treated with 50% formamide and electrophoresed on a 1.3% agarose gel containing 2.2 M formaldehyde. Blotting and autoradiography were carried out as described in section 2. Size markers are in nucleotides. (A) Frontal cortex, (B) cerebellum (20 µg per lane).

Fig.2. Developmental changes of CCK mRNA in rat brains. Cytoplasmic RNA from developing rat brains, fetal (F) and postnatal (1–90 days), were spotted onto a nitrocellulose membrane and hybridized with $^{32}$P-CCK cDNA as described in section 2. The concentration of cytoplasmic RNA was 10 µg.
Fig. 3. Regional distribution of CCK mRNA in rat brains. The cytoplasmic RNA from various regions of rat brains was analyzed by dot-blot hybridization using \(^{32}\)P-CCK cDNA. The concentrations of cytoplasmic RNA were 12.5 (1), 6.25 (2), 3.13 (3) and 1.56 \(\mu\)g (4).

These results may suggest that CCK concentration is mainly transcriptionally regulated and possesses an intimate relationship with differentiation and maturation of the neuron in the brain.

3.3. Regional distribution of CCK mRNA in adult rat brain

Dot-blot analysis showed that the CCK mRNA level of the frontal cortex was highest, followed by those of the hippocampus, striatum and cerebellum (fig.3). The levels in the striatum and hippocampus were almost the same. This result is also in agreement with our data about the regional distribution of CCK in rat brains (not shown). Beinfeld et al. [13] and Dockray [2] reported similar findings on CCK content. It may be important that the frontal cortex contains the highest CCK mRNA. This result may indicate that CCK plays an important role in mental function. The CCK precursor may be synthesized and processed to oligopeptides in the neurons of the frontal cortex, hippocampus and striatum, and transported to the axon and synapses by axonal flow. CCK may act there as a neurotransmitter or a neuromodulator. We are currently trying to determine the exact cellular localization of CCK mRNA by in situ hybridization.

REFERENCES