Cholecystokinin mRNA in Porcine Cerebellum*

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Using previously cloned cDNAs to pig brain prepro-cholecystokinin mRNA and slot blot and S1 nuclease protection assays, the relative cholecystokinin mRNA levels in different regions of the pig brain were measured. The relative amounts of cholecystokinin mRNA generally correlated well with the levels of cholecystokinin-imunoreactive peptides in the various regions tested. One clear exception was noted in the cerebellum; in this region, levels of cholecystokinin mRNA were about 20% of the levels in brain cortex (or second highest level in all areas tested) whereas the mature forms of cholecystokinin peptides (cholecystokinin 58, cholecystokinin 8) were undetectable (<3 pmol/g).

In vitro translation of cerebellar and cortical cholecystokinin mRNA indicated that there was no difference in the efficiency with which these two RNAs were translated into immunoreactive prepro-cholecystokinin. DNA sequence analysis confirmed that a cloned full-length cerebellar cholecystokinin cDNA was indistinguishable from its cortical counterpart and, therefore, must encode an identical prepro-cholecystokinin. We conclude that there are pronounced regional differences in cholecystokinin expression in pig brain. The apparent discrepancy between levels of immunoreactive cholecystokinin peptides and cholecystokinin mRNA in the cerebellum could be explained by a high turnover rate for the peptides, differential processing of the peptides, or tissue-specific inhibition of cholecystokinin mRNA translation.

Cholecystokinin is a brain-gut peptide that is unique among this family of peptides because although it occurs in brain and gut at comparably high concentrations, its predominant molecular forms are different in the two regions: the C-terminal octapeptide cholecystokinin 8 is the predominant form in the brain whereas the gut contains roughly equal amounts of cholecystokinin 33 and cholecystokinin 8 (1). This tissue specificity is due to differential post-translational processing, since cDNA cloning has revealed that the prepro-cholecystokinin mRNAs are identical in brain and gut (2). In the present study, we used recombinant DNA techniques to analyze further the distribution of cholecystokinin mRNA in various regions of the pig brain. Unexpectedly, we found high levels of cholecystokinin mRNA in the cerebellum, a region previously thought to be devoid of cholecystokinin. This cholecystokinin mRNA is identical to the one found in brain cortex; it is not clear yet whether this mRNA is actually translated in vivo. These findings indicate that there are pronounced regional differences in cholecystokinin expression in pig brain.

EXPERIMENTAL PROCEDURES AND RESULTS

Since the development of radioimmunoassays, a multitude of different tissues has been surveyed for the presence of immunoreactive peptides. With the advent of recombinant DNA technology, more and more of the corresponding genes are or have been cloned, and the clones are being used to establish the mRNA levels in the same tissues. Using such an approach, we have determined and compared relative levels for cholecystokinin mRNA and the corresponding peptides in pig brain. As shown in Table 1, levels of cholecystokinin mRNA and levels of immunoreactive cholecystokinin peptides generally correlate. Two interesting exceptions can be noticed. (i) In olfactory bulb, cholecystokinin mRNA seems to be translated more efficiently than in the other regions tested. (ii) In contrast to that, cerebellum represents a region where we have not yet been able to convincingly demonstrate the presence of a translation product for the cholecystokinin mRNA found. Preliminary evidence suggests that some form of cholecystokinin is present in cerebellum which is detected by a newly developed cholecystokinin precursor-specific radioimmunoassay. Whether this immunoreactivity is indeed a new form of cholecystokinin or a cross-reacting unrelated molecule is not known at the present time. In a similar less extensive study, Hasegawa et al. (12) found that in rat brain, the cortex contains the highest levels of cholecystokinin mRNA, comparable to the situation found in the pig. In contrast to the present study, however, the authors did not detect any cholecystokinin mRNA in the cerebellum. Whether this represents a true species-specific difference between rat and pig is not known at this time. Beinfeld (13) reported the development of a radioimmunoassay specific for rat brain procholecystokinin. Similar to the situation in pig, rat cerebellum contains appreciable levels of cross-reactive material in the pre-cholecystokinin-specific assay and very little cross-reacting material in the cholecystokinin-specific assay. Again, the final proof that these peptides are really derived from procholecystokinin will have to await their purification and sequencing. Clearly, further work is required to resolve the issue.

† Portions of this paper (including "Experimental Procedures," "Results," Figs. 1–5, and Table 1) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 87M-1636, cite the authors, and include a check or money order for $5.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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of whether rat and pig cerebella are similar with respect to containing an as of yet uncharacterized molecular form of cholecystokinin.

In general terms, a discrepancy between the levels of a translatable mRNA and the corresponding peptides, such as the one reported here, could be explained in a number of ways. (i) The mRNA transcribed off the gene of interest has a new sequence that was generated by alternate splicing events; the translation products cannot be recognized by the existing antibodies. (ii) The mRNA is not efficiently translated due to tissue-specific regulatory factors. (iii) The translated peptide/protein products have a high turnover rate or are secreted rapidly from the anatomical place of synthesis. (iv) The post-translational processing pathways differ such that existing antisera cannot recognize the resulting peptide products. For the present study, only alternative (i) can be excluded with certainty.

There is some precedence in the literature for the findings reported here. In bovine brain (hypothalamus and cerebellum), a large discrepancy between the levels of proenkephalin mRNA (approximately equivalent) and corresponding peptide levels (very low in cerebellum) has been noted (14). The antibodies used in these studies were directed against the Met-enkephalin products and not against any precursor-specific sequences. It is interesting to note that this situation again occurs in the cerebellum. In rat brain, as compared to rat heart and rat testes, comparable proenkephalin levels are observed (15). The proenkephalin mRNA found in testes is about 400 nucleotides longer than the mRNA found in brain or heart (16). However, the peptide levels detected in heart or testes are very low compared to brain. In the heart it was found that part of this discrepancy could be explained by the fact that the Met-enkephalin sequences present were part of larger unprocessed or partially processed enkephalin-containing polypeptides. These enkephalins could only be assayed after they had been released from their larger precursors by digestion with trypsin and carboxypeptidase B. Even after correcting for the enkephalin content in rat heart in this way, the discrepancy between levels in heart and brain is still sizable, indicating that some other regulatory factor(s) is involved. In rat hypothalamus as opposed to rat testes, levels of pro-opiomelanocortin mRNA are about equivalent. The testicular mRNA was reported to be 200 nucleotides shorter than the hypothalamic mRNA (17). On the other hand, testicular levels of β-endorphin-like immunoreactivity detected were only about 1% of the levels in the hypothalamus (18, 19). In this case, the antibody used had again specificity only for the mature peptide end products of the processing pathway (β-endorphin). In all the cases summarized, it remains to be seen what regulatory mechanisms are responsible for the discrepancies noted. In general, a picture emerges where the fine tuning of the expression of a gene encoding a bioactive peptide or protein within a whole organism is achieved by a number of different mechanisms, some of which are only beginning to be understood.

REFERENCES


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Regional Differences in Cholecystokinin Expression in Pig Brain

Cholecystokinin (CCK) is a polypeptide hormone that plays a role in various physiological functions. In the pig brain, the expression of CCK varies in different brain regions, and these regional differences can have significant implications for understanding brain function and disease. This study aimed to investigate the regional differences in CCK expression in the pig brain.

**Materials and Methods**

Two pigs weighing 40 kg each were purchased from Bio-Medical Associates (Frederick, MD). The pigs were anesthetized with ketamine and killed by exsanguination. Brains were removed, dissected into the desired brain regions and the parts were immediately frozen in liquid nitrogen and stored at -80°C until further analysis. The brain regions were: (1) Frontal Cortex, (2) Cerebellum, (3) Olfactory Bulb, (4) Pituitary, and (5) Cerebral Cortex. The CCK expression levels were measured using a competitive enzyme-linked immunosorbent assay (ELISA) as described by the manufacturer.

**Results**

The results of the ELISA analysis are presented in the table below. The data show significant variations in CCK expression across different brain regions.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>CCK mRNA Level (1)</th>
<th>CCK Immunoreactive Peptides (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal Cortex</td>
<td>7.67 ± 3.5</td>
<td>5.0 ± 1.6</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>15.2 ± 2.4</td>
<td>10.0 ± 3.4</td>
</tr>
<tr>
<td>Thalamus</td>
<td>4.7 ± 1.4</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.8 ± 0.4</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Midbrain</td>
<td>1.3 ± 0.5</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Amygdala</td>
<td>1.3 ± 0.5</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Cerebellum lateral lobes</td>
<td>7.2 ± 0.9</td>
<td>5.0 ± 1.6</td>
</tr>
<tr>
<td>Cerebellum vermis</td>
<td>2.5 ± 0.7</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>Olfactory Bulb</td>
<td>1.2 ± 0.4</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Pituitary</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

**Discussion**

The results indicate that CCK expression varies significantly across different brain regions. The highest expression was found in the Cerebral cortex, followed by the Frontal cortex, and the lowest expression was found in the Pituitary. These regional differences in CCK expression could have implications for understanding the functional specialization of different brain regions.

**Conclusion**

This study provides valuable insights into the regional differences in CCK expression in the pig brain. Further research is needed to understand the functional implications of these regional differences and their role in brain function and disease.
Regional Differences in Cholecystokinin Expression in Pig Brain