Chromosomal Localization of the Gastric and Brain Receptors for Cholecystokinin (CCKAR and CCKBR) in Human and Mouse

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Abstract

Receptors for cholecystokinin (CCK) can be pharmacologically classified into at least two distinct subtypes, CCK_AR and CCK_BR. In an effort to determine whether the CCK_AR and CCK_BR receptors may be associated with certain CNS or gastrointestinal diseases, we have localized and compared the human and mouse chromosomal loci encoded by the CCKAR and CCKRR genes. The gene encoding the CCK_AR receptor maps to a syntenic region of human chromosome 4 and mouse chromosome 5. The CCK_BR receptor gene, on the other hand, resides on a syntenic region of human chromosome 11 and distal mouse chromosome 7. Localization of the CCK receptors with two dopamine receptors, DRD5 (4p15.1–15.3) and DRD4 (11p15), provides the interesting possibility of coinvolve in neuropsy-chiatric or CNS illnesses.
(corresponding to the human genomic fragment) was clearly seen in the human control lane upon hybridization with this probe, but no CCK_A receptor-specific hybridization signal was detectable in any of the human-hamster hybrid lanes (data not shown). This result could be due to the low representation of a specific human chromosome in this panel. Therefore, we performed a PCR assay using sequence-specific primers for human CCK_A receptor (1121S-GAACAACGCTTCCGCCTCGG; A-3' -AS-CGTTCTTTTCTCTCTGCG-TCC) on the same panel of human-hamster hybrid DNAs. Two hybrids, 803 and 1006, scored positive for the human CCK_A receptor in the PCR assay. We conclude that the human receptor for CCK_A, CCKAR, resides on human chromosome 4 since this is the only human chromosome retained in both hybrids 803 and 1006 (data reviewed, but not shown).

To map the receptor for CCK_A in the mouse, we utilized a wild by inbred backcross panel of mice [(BALB/cAn × Mus spretus) F_1 × BALB/cAn] that has been previously typed for a large number of DNA markers spanning the mouse genome. Initially, an MspI RFLP was detected between the parental strains BALB/c and M. spretus with the CCK_A receptor probe (Fig. 1). The segregation of the CCK_A receptor MspI RFLP was then compared with previously typed DNA markers among 72 backcross mice. The most likely position of the receptor for CCK_A was found to reside 11.1 ± 3.7 cM distal to the marker D5Mit4 on mouse chromosome 5 (Fig. 2). This region of mouse chromosome 5 corresponds to a syntenic region of human chromosome 4p16.2–p15.1 (11), also consistent with our positioning of CCKAR on human chromosome 4.

We determined the chromosomal location of the receptor for CCK_B by hybridization of a human CCK_B receptor cDNA probe to a Southern filter containing BamHI-digested DNAs from the human–hamster hybrid panel. A single 11-kb BamHI fragment was detected in the human control lane as well as in the hybrid 1049 (data not shown). To confirm this result, we amplified the panel of human-hamster hybrid DNAs by PCR using human-specific CCK_B receptor primers (781S—GTGGTTGGCTCAGTTTATAG; 466AS–GCTTTGGGT-GTTGGTTTCTCTGT). A PCR-amplified product specific to human DNA was found in two hybrids, 803 and 1049, as well as in the human control, consistent with human chromosome 11 (data reviewed, but not shown).

The receptor for CCK_B was also positioned in the mouse by utilizing the (BALB/cAn × M. spretus) F_1 × BALB/cAn backcross panel of mice. The DNA probe for CCK_B receptor was hybridized to digested DNA from each parental strain, and several RFLPs were identified, including EcoRI, PstI, SacI, EcoRV, and PvuII (data not shown). The SacI RFLP was utilized since only a single hybridizing fragment was observed in each parental strain, thus ensuring the identification of the structural Cckbr locus in the mouse (Fig. 1). The SacI RFLP was followed among 71 backcross individuals and permitted us to place the Cckbr locus approximately 12.6 ± 3.9 cM distal to the MIT marker D7Mit7 and 12.6 ± 3.9 cM proximal to Pkcb on mouse chromosome 7 (Fig. 2). The location of Cckbr in this region places this locus in a region of known synteny with regions on the short and long arms of human chromosome 11 (2), which is consistent with our chromosome 11 location for CCK_B receptor. Recently, the human locus for CCK_B has also been determined to reside at chromosome 11p15.4 by fluorescence in situ hybridization (21).
It is most intriguing that the locations for both receptors of CCK correspond closely to the positions assigned to other members of the G-protein-coupled receptors, the dopamine family of receptors. For example, we find that CCKAR and the previously mapped dopamine receptor DRD5 (19) both appear to reside on human chromosome 4p15.1–p15.3. Similarly, CCKBR and another dopamine receptor, DRD4, appear to colocalize to human chromosome 11p15.4 (6, 16). Thus, a foundation for possible coassociation of G-coupled receptor genes appears to exist on two regions of human chromosomes 4 and 11.

The dopamine family of receptors is thought to have diverged from a single ancestor into a group of five homologous genes referred to as D1–D5 [for review see (22)]. Dopamine and its receptors play a major role in regulating motor activity principally through the D1 and D2 receptors present in the neostriatum and affect behavior through all five subtypes found in the nucleus accumbens, olfactory tubercle, frontal cortex, and amygdala. Similar to dopamine receptors, CCK receptors are expressed in distinctive patterns. The CCKA receptors predominate in the periphery, where they mediate pancreatic secretion and gallbladder motility. The CCKA receptors are also present in the vagus nerve and select areas of the CNS (interpeduncular nucleus, solitary complex, hypothalamus, and nucleus accumbens). The CCKB receptor, on the other hand, predominates in the CNS, where it is widely distributed throughout the brain. Thus, different regulatory signals must act in a tissue-specific fashion for receptors of CCK and dopamine. For example, it is known that regulation plays a role in the coexistence of CCK and dopamine-containing neurons (ca. 40%) in the ventral mesencephalon, which project to the medial nucleus accumbens, septum, and olfactory tubercle in rats and primates. These mesolimbic CCK-containing dopaminergic neurons and their terminal projections regulate behavior, emotion, mood, and motivation and thus are implicated in schizophrenia, Parkinson disease, anxiety, and drug addiction.

On the basis of the similarity in expression patterns, biological effects, and the colocalization in the human genome, we propose that receptors for cholecystokinin and dopamine may possibly interact with one another, perhaps via coregulation. Evolutionarily, these receptors may have coevolved, and we might predict that other G-coupled proteins may also reside close to these loci. In fact, the m4 muscarinic cholinergic receptor has recently been found to reside on human chromosome 11p11–12 (7). It will now be of interest to compare regulatory regions of these closely associated genes to determine whether colocalization also applies to coregulation.

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FIG. 1.
CCK\textsubscript{AR}− and CCK\textsubscript{BR}-specific RFLPs in the mouse. A Southern hybridization of MspI-digested (CCKAR, \textbf{left}) or Sacl-digested (CCKBR, \textbf{right}) DNAs from BALB/cAn (C), \textit{M. spretus} (S), or (BALB/cAn × \textit{M. spretus}) F\textsubscript{1} (C/S). The DNA probe corresponding to the receptor for CCK\textsubscript{A} is a 2.2-kb human cDNA clone (SW. and J.R.P., unpublished). The DNA probe corresponding to the receptor for CCK\textsubscript{B} is a human 1.9-kb cDNA (17). Final wash stringency was 0.2× SSC at 55°C.
FIG. 2.
Pedigree analysis of polymorphic DNA markers among (BALB/cAn × M. spretus) F1 × BALB/cAn progeny for mouse chromosomes 5 (top) and 7 (bottom). The loci typed in the cross are indicated on the left. Each column represents the type of chromosome identified in the panel, and the number of progeny is listed at the bottom. The RFLP used and the appropriate size differences detected between BALB/cAn (C) and M. spretus (S) are indicated on the right. SSLP refers to simple sequence length polymorphism used according to Dietrich et al. (4). The distance (cM ± standard error) between markers is indicated at the bottom.

### Marker analysis for chromosome 5

| Marker | C | S |
|--------|---|---|
| If-6   | 4.2 | 10.0 |
| D5Mit4 | 3.0 | 3.9,2.4 |

RFLP: Sac I, SSLP, Msp I

### Marker analysis for chromosome 7

| Marker | C | S |
|--------|---|---|
| Bcl-3  | 2.8 | 3.0 |
| D7Mit17| 5.5 | 3.0 |
| Cckbr  | 9.0 | Barn HI |

RFLP: Eco RI, SSLP, Sac I