SHORT COMMUNICATION

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

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Receptors for cholcystokinin (CCK) can be pharmacologically classified into at least two distinct subtypes, CCK_AR and CCK_BR. In an effort to determine whether the CCK_A and CCK_B 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 CCKBR genes. The gene encoding the CCK_A receptor maps to a syntenic region of human chromosome 4 and mouse chromosome 5. The CCK_B 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–p15.3) and *DRD4* (11p15), provides the interesting possibility of coinvolvement in neuropsychiatric or CNS illnesses.

The cholecystokinin family of peptides and their receptors are widely distributed in the gastrointestinal and central nervous systems. The receptors for cholecystokinin can be pharmacologically classified into two subtypes, CCK_A and CCK_B, on the basis of their affinity for nonsulfated analogues of CCK and recently developed subtype-specific agonists (1, 13, 15, 18). CCK_A receptors are predominantly found in the gastrointestinal tract, where they mediate pancreatic secretion, motility, and growth and are present in select nuclei of the central nervous system (5, 9, 10). CCK_B receptors are found throughout the central nervous system, where they regulate anxiety, satiety, analgesia, and neuroleptic activity (3, 13, 20, 25). We have recently cloned both the CCK_A and the CCK_B receptors from rat and human (17, 23, 24). These receptors share 48% homology at the amino acid level and at present are the only known members of the cholecystokinin receptor family. On the structural basis of their seven hydrophobic transmembrane domain motifs (17, 23, 24) as well as their modulation by guanine nucleotides (12, 14), they constitute a subfamily of the guanine nucleotide-binding regulatory protein-coupled superfamily of receptors.

To characterize further the CCK family of receptors, we have determined the chromosomal positions of the genes for both the CCK_A and CCK_B receptors in human and mouse. Since the cDNA corresponding to the human CCK_A receptor has been cloned and sequenced, we initially utilized this cDNA probe to screen a panel of human–hamster hybrid DNAs (Bios Inc.) for localization to a specific human chromosome. A 20-kb *Bam*HI fragment (corresponding to the human genomic fragment) was clearly seen in the human control lane upon hybridization with this probe, but no CCK_AR-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 CCKAR (1121S—GAACAAACGCTTCCGCCTCGG; A-3′-AS—CGTTCTTTCTTCTCTGCCTCC) 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 *Msp*I RFLP was detected between the parental strains BALB/c and *M. spretus* with the CCK_AR probe (Fig. 1). The segregation of the CCK_AR *Msp*I 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_BR cDNA probe to a Southern filter containing *Bam*HI-digested DNAs from the human–hamster hybrid

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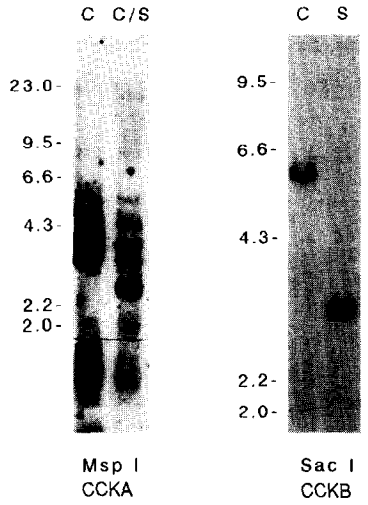

FIG. 1. CCK_AR- and CCK_BR-specific RFLPs in the mouse. A Southern hybridization of *Msp*I-digested (CCKAR, **left**) or *Sac*I-digested (CCKBR, **right**) DNAs from BALB/cAn (C), *M. spretus* (S), or (BALB/cAn × *M. spretus*) F_1 (C/S). The DNA probe corresponding to the receptor for CCK_A is a 2.2-kb human cDNA clone (S.W. and J.R.P., unpublished). The DNA probe corresponding to the receptor for CCK_B is a human 1.9-kb cDNA (17). Final wash stringency was 0.2× SSC at 55°C.

panel. A single 11-kb *Bam*HI 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_BR primers (781S—GTGTTGGTTGCCAGTTTATAG; 466AS—GCTTTGGGTGTTGGTTTCCTG). 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_BR was hybridized to digested DNA from each parental strain, and several RFLPs were identified, including *Eco*RI, *Pst*I, *Sac*I, *Eco*RV, and *Pvu*II (data not shown). The *Sac*I 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 *Sac*I 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 *D7Mit17* 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 *CCKBR*. Recently, the human locus for *CCKBR* 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 CCK_A receptors predominate in the periphery, where they mediate pancreatic secretion and gallbladder motility. The CCK_A receptors are also present in the vagus nerve and select areas of the CNS (interpeduncular nucelus, solitary complex, hypothalamus, and nucleus accumbens). The CCK_B 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 dopamin-

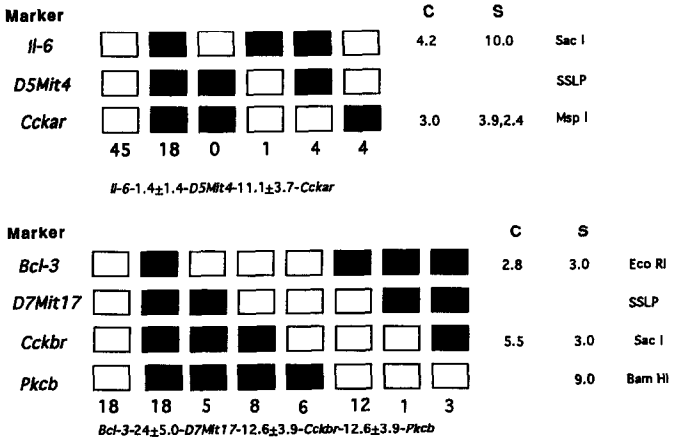

FIG. 2. Pedigree analysis of polymorphic DNA markers among (BALB/cAn × *M. spretus*) F_1 × BALB/cAn progeny for mouse chromosomes 5 (**top**) and 7 (**bottom**). The loci typed in the cross are indicated on the left. Each column represents the type of chromosome identified in the panel, and the number 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.

ergic 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–p12 (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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