



Presence and distribution of motilin and motilin receptors in the brain of the rabbit

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Abstract

AIM: To identify and sequence motilin mRNA from the brain and to study the distribution of motilin and its receptor in the brain. In view of our findings the effect on food intake was explored. The presence of motilin immunoreactivity in the brain has been debated. Recently we reported motilin receptors in the cerebellum.

METHODS: Total RNA was prepared from different regions of the rabbit brain. Single-stranded cDNA was synthesized using superscript II RT and PCR was performed with TAQ DNA polymerase and specific primers. Bands of ± 300 bp, ± 500 bp and ± 1000 bp were obtained in all regions examined. Hybridization with a 32 P human cDNA probe confirmed that the 300 bp band was motilin. PCR products were cloned into PCR TMII vector and screened with the 32 P probe. The nucleotide sequence obtained from positive clones showed 7 substitutions, resulting in 4 amino acid differences, compared to the rabbit duodenal motilin precursor. The amino acid

substitutions were in the signal peptide [-11 (Val→Ala)] and the motilin associated peptide [33 (Asp→Glu), 34 (Ala→Pro), 52 (Met→Thr)]. The concentration of motilin, measured by radioimmunoassay, was significantly higher in extracts from hippocampus (3780 ± 309) and cerebellum (2114 ± 121), than in the medulla (1239 ± 39) (All data in fmol/mg protein). Autoradiographic studies with 125 I motilin on rabbit coronal brain sections revealed specific binding in several regions of the brain. Binding studies with homogenates identified two binding sites (pK_d , $hi = 9.03$, pK_d , $lo = 6.65$) in each of these regions but the density of high affinity binding sites (fmol/mg protein) was significantly lower in the hippocampus (1.7 ± 0.66), prefrontal lobe (1.3 ± 0.5), olfactory tract (0.9 ± 0.3) and thalamus (3.5 ± 0.9) than in the cerebellum (8 ± 2). The effect of the *i.v.* administration of motilin ($10 \mu\text{g/kg}$) on food intake was studied in rabbits deprived from food for 2 d. Motilin significantly stimulated food intake during the first hour with $9.6\% \pm 0.4\%$ compared to control rabbits, while bombesin ($10 \mu\text{g/kg}$) decreased food intake with $16\% \pm 1.3\%$. The effect persisted after two hours.

CONCLUSION: Motilin is present in the brain, but the motilin gene shows polymorphism. The motilin receptor is present in different regions. Our data suggest motilin is a neuropeptide, with possible central effects.

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