Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Generation and characterization of hD_5 and C-terminal Mutant hD_{5m} transgenic rats

Zhiliang Xu[a,b,1], *Suzhen Dong*[a,b,1], *Dan Du*[b], *Nan Jiang*[b], *Peiqua Sun*[c], *Huikun Wang*[c], *Liang Yin*[b], *Xuliang Zhang*[b], *Xiaohua Cao*[b], *Xuechu Zhen*[c], *Yinghe Hu*[a,*]

[a]*Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, East China Normal University, Shanghai 200062, PR China*

[b]*Key Laboratory of Brain Functional Genomics, Ministry of Education, East China Normal University,Shanghai 200062, PR China*

[c]*Department of Pharmacology, College of Pharmacy, Soochow University, Suzhou 215123, PR China*

ARTICLE INFO

Article history:
Accepted 28 January 2012
Available online 4 February 2012

Keywords:
D_1-like dopamine receptor
Learning and memory
Transgenic rat
LTP

ABSTRACT

Dopamine D_1-like receptors play important roles in many brain activities such as cognition and emotion. We have generated human hD_5 and mutant human hD_5 (hD_{5m}) transgenic rats. The C-terminal juxtamembrane domain of mutant hD_5 was identical to that of hD5 pseudogenes. The transgenes were driven by the CAMKII promoter that led the expression mainly in the cerebral cortex and hippocampus. We have used different dopamine receptor agonists to compare the pharmacological profiles of the human hD_5 and hD_{5m} receptors. The results showed that they exhibited distinct pharmacological properties. Our results of pharmacological studies indicated that the C-terminal of D_5 receptor could play important roles in agonist binding affinity. Hippocampal long-term potentiation (LTP) evoked by tetanic stimulation was significantly reduced in both transgenic rats. In addition, we found that the overexpression of dopamine hD_5 and hD_{5m} receptors in the rat brain resulted in memory impairments. Interestingly, an atypical D_1-like receptor agonist, SKF83959, could induce anxiety in hD_{5m} receptor transgenic rats but had no effect on the anxiety-like behavior in D_5 receptor transgenic and wild-type rats.

1. Introduction

Dopamine has been shown to play important roles in many brain functions including cognition, locomotion activity and emotion (Missale et al., 1998; Vallone et al., 2000). The physiological activities of dopamine are mediated via a family of G-protein coupled receptors (GPCRs). The dopamine receptors are generally divided into two groups, namely the D_1-like and D_2-like receptors. Pharmacologically, D_5 receptor exhibits the classical ligand-binding characteristics of D_1-like receptors, and displays a 10-fold higher affinity than D_1 receptor for the endogenous ligand dopamine (Sunahara et al., 1991).

Since the dopamine D_1 and D_5 receptors are pharmacologically undistinguishable, it is not clear which receptor subtype participates in the regulation of cognition and mood functions (Castellano et al., 1991; Hotte et al., 2005). It is interesting to note that the tissue distribution patterns of D_1 and D_5 receptors are very different. D_1 receptor is widely distributed in many

* *Corresponding author*. Fax: +86 21 62601953.
E-mail address: yhu@brain.ecnu.edu.cn (Y. Hu).
[1] Co-first author with the same contribution to this study.

0006-8993/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2012.01.076

tissues, while D_5 receptor is mainly expressed in the brain (Beischlag et al., 1995; Ciliax et al., 2000; Khan et al., 2000). Moreover, the highest level of D_5 expression is restricted in the cortex and hippocampus (Khan et al., 2000). Since these two brain regions have been strongly implicated in a variety of learning and memory processes, we speculated that the dopamine D_5 receptor subtype might participate in the regulation of learning and memory. Recently, a number of genetic engineered mouse models have been generated for the studies on the physiological functions of D_1-like receptors. These studies demonstrated that D_1-like receptors could be involved in hypertension (Jiang et al., 2003), elevation of sympathetic tone (Hollon et al., 2002), locomotion, startle, and prepulse inhibition (Holmes et al., 2001).

In this report, we demonstrated that human D_5 receptor is involved in memory. We have isolated a mutant hD_5 dopamine receptor gene (named hD_{5m}) in which the hD_5 carboxyl terminal was replaced with C-terminal juxtamembrane domain of hD_5 pseudogene (Fig. 1). It has been reported that the C-terminal of D5 receptor was involved in interaction with GABA-A receptor (Liu et al., 2000). Therefore, we used the C-terminal mutant receptor to explore whether the mutation could affect the receptor function. Our pharmacological studies showed that the C-terminal of hD_5 receptor was involved in the binding affinity with its agonist. Transgenic rats expressing human D_5 and the mutant hD_{5m} in forebrain have been generated to study the physiological functions of D_5 receptor and its carbonyl terminal. Electrophysiological analysis showed that hippocampal long-term potentiation (LTP) evoked by tetanic stimulation was significantly reduced in both hD_5 and hD_{5m} transgenic rat. Furthermore, we found that the overexpression of dopamine hD_5 and hD_{5m} genes in the brain resulted in memory impairments, indicating that dopamine D_5 receptor may play important roles in cognitive functions in rat. Our results also demonstrated that the mutant C-terminal of D_5 was involved in anxiety behavior.

2. Results

2.1. *Pharmacological characterization of the C-terminal mutant of hD_5 dopamine receptor*

We have isolated a mutant human dopamine D_5 receptor (hD_{5m}) using PCR method. DNA and protein analyses showed that the C-terminal region of the mutant receptor was identical to the hD_5 pseudogene (Fig. 1). We have performed radioligand binding assay, cAMP assay and calcium image to investigate the pharmacological differences between hD_5 and hD_{5m}. Saturation radioligand binding analysis using [^{3}H]SCH23390, a radiolabeled D_1-like receptor antagonist, showed that the receptor densities (B_{max}) in the membrane preparations of h_{D5} and hD_{5m} cell lines were almost identical (hD_5, 0.61±0.03 pmol/mg; hD_{5m}, 0.69±0.03 pmol/mg). However, the affinity (K_d) for [^{3}H] SCH23390 binding to the hD_{5m} receptor was significantly different from that of hD_5 receptor (hD_5, 1.19±0.16 nM; $hD5_m$, 2.37± 0.25 nM. $p<0.01$) (Fig. 2A&B and Table 1). We further analyzed the pharmacological properties of hD_5 receptors and hD_{5m} using two D_1-like receptor agonists SKF83959 and SKF38393. As shown in Fig. 3 and Table 1, the K_i value of SKF83959 for hD_{5m} was seven-fold higher than that for hD_5 receptor (hD_5, 3.09±0.81 nM; $hD5_m$, 21.08±4.87 nM). On the other hand, SKF38393 for hD_5 receptor were only two-fold lower than that for hD_{5m} (hD_5, 0.53±0.04 μM; hD_{5m}, 1.36±0.32 μM), indicating the different binding property of the two D_1-like receptor agonists on hD_5 receptor and hD_{5m}.

We further examined the biological activity of the mutant dopamine receptor in a cell-based assay. cAMP accumulation stimulated by SKF38393 and SKF83959 did not exhibit any significant difference between hD_5 and hD_{5m} (Fig. 3 and Table 1). Since recent reports indicated that hD_5-like receptor activation could induce the production of intracellular calcium release (Lezcano and Bergson, 2002; Tang and Bezprozvanny, 2004), we examined the potential effect of hD_5 and hD_{5m} activation on intracellular

```
Protein  381 Arg Thr Pro Val Glu Thr Val Asn Ile Ser Asn Glu Leu Ile Ser Tyr Asn Gln Asp Met    400
D5m     1141 cgc acg ccg gtg gag acg gtg aac atc agc aat gag ctc atc tcc tac aac  caa gac atg   1200
D5                                                                              c(Ile)
D5ψ

Protein  401 Val Phe His Lys Glu  Ile  Ala Ala Ala Cys Ile His Met Met Pro Asn Ala Leu Pro Pro   420
D5m     1201 gtc  ttc  cac aag gaa atc gca gct gcc tgc atc cac atg  atg ccc  aac gcc ctt  ccc ccc 1260
D5                                      a(Tyr)                                  a(Thr)
D5ψ

Protein  421 Gly  Asp  Gln  Glu Val AspAsnAsp Glu Glu Glu Glu Ser ProPheAspArg MetPheGln   440
D5m     1261 ggg gac   caa gag gtg gac aac gat gag gag gag gag agt cct ttc gat cgc  atg ttc cag 1320
D5             c a(Asn)gg(Glu)                 c          ---       g(Gly)
D5ψ                                                                             c

Protein  441  Ile Tyr Gln Thr Ser Pro Asp Gly Asp Pro Val Ala Glu Ser Val Trp Glu Leu Asp Cys   460
D5m     1321  atc tat cag acg tcc cca gat  ggt  gac cct  gtt  gct  gag tct  gtc  tgg gag ctg  gac tgc  1380
D5
D5ψ                        a                              a              a              g

Protein 461  Glu Gly Glu Ile Ser Leu Asp Lys Ile  Thr  Pro Phe Thr Pro Asn Gly Phe His          479
D5m    1381`gag ggg gag att tct  tta  gac  aaa ata aca cct  ttc  acc ccg aat  gga ttc  cat taa act  1440
D5
D5ψ                                        g                 a
```

Fig. 1 – Comparison of nucleotide and deduced amino acid sequences of C-terminals of hD_5, hD_{5m} and pseudogenes $hD_{5\Psi}$. The different base pairs are shown between hD_5, hD_{5m} and the pseudogene. Deletions and insertions are presented with dash and expanded carets.