Available online at www.sciencedirect.com
ScienceDirect
Neurobiology of Learning and Memory 87 (2007) 42–56
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

Working memory deficits in transgenic rats overexpressing human adenosine A_{2A} receptors in the brain

Lydia Giménez-Llort [a], Serge N. Schiffmann [b], Tanja Shmidt [c], Laia Canela [d], Lluïsa Camón [e], Monica Wassholm [f], Meritxell Canals [f], Anton Terasmaa [f], Albert Fernández-Teruel [a], Adolf Tobeña [a], Elena Popova [c], Sergi Ferré [g], Luigi Agnati [h], Francisco Ciruela [d], Emili Martínez [e], Jörgen Scheel-Kruger [i], Carmen Lluis [d], Rafael Franco [d], Kjell Fuxe [f,*], Michael Bader [c,*]

[a] *Medical Psychology Unit, Department of Psychiatry and Forensic Medicine, School of Medicine, Institute of Neuroscience, Autonomous University of Barcelona, Barcelona, Spain*
[b] *Laboratory of Neurophysiology CP601, Université Libre de Bruxelles, Brussels, Belgium*
[c] *Max-Delbrück-Center for Molecular Medicine (MDC), D-13125 Berlin-Buch, Germany*
[d] *Institute of Biomedical Investigations of Barcelona, CSIC, IDIBAPS, Barcelona, Catalonia, Spain*
[e] *Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Catalonia, Spain*
[f] *Department of Neuroscience, Karolinska Institute, Stockholm, Sweden*
[g] *Behavioural Neuroscience Branch, National Institutes of Health, National Institute on Drug Abuse, Baltimore, MD, USA*
[h] *Department of Biomedical Sciences, University of Modena, Modena, Italy*
[i] *Department of Behavioural Pharmacology, NeuroSearch A/S, Ballerup, Denmark*

Received 29 March 2006; revised 23 May 2006; accepted 25 May 2006
Available online 7 July 2006

Abstract

Adenosine receptors in the central nervous system have been implicated in the modulation of different behavioural patterns and cognitive functions although the specific role of A_{2A} receptor ($A_{2A}R$) subtype in learning and memory is still unclear. In the present work we establish a novel transgenic rat strain, TGR(NSEhA2A), overexpressing adenosine A_{2A}Rs mainly in the cerebral cortex, the hippocampal formation, and the cerebellum. Thereafter, we explore the relevance of this A_{2A}Rs overexpression for learning and memory function. Animals were behaviourally assessed in several learning and memory tasks (6-arms radial tunnel maze, T-maze, object recognition, and several Morris water maze paradigms) and other tests for spontaneous motor activity (open field, hexagonal tunnel maze) and anxiety (plus maze) as modification of these behaviours may interfere with the assessment of cognitive function. Neither motor performance and emotional/anxious-like behaviours were altered by overexpression of A_{2A}Rs. TGR(NSEhA2A) showed normal hippocampal-dependent learning of spatial reference memory. However, they presented working memory deficits as detected by performance of constant errors in the blind arms of the 6 arm radial tunnel maze, reduced recognition of a novel object and a lack of learning improvement over four trials on the same day which was not observed over consecutive days in a repeated acquisition paradigm in the Morris water maze. Given the interdependence between adenosinic and dopaminergic function, the present results render the novel TGR(NSEhA2A) as a putative animal model for the working memory deficits and cognitive disruptions related to overstimulation of cortical A_{2A}Rs or to dopaminergic prefrontal dysfunction as seen in schizophrenic or Parkinson's disease patients.

Keywords: Adenosine receptors; Dopamine receptors; Transgenic rat; Memory; Prefrontal cortex; Heterodimerization

* Corresponding authors. Fax: +46 8 337941 (K. Fuxe), +49 30 9406 2110 (M. Bader).
E-mail addresses: Kjell.Fuxe@neuro.ki.se (K. Fuxe), mbader@mdc-berlin.de (M. Bader).

1074-7427/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2006.05.004

1. Introduction

Adenosine A_{2A} receptors are thought to play a role in a number of physiological responses and pathological conditions (reviewed by Moreau & Huber, 1999; Popoli et al., 2003). Their high expression in the striatum and antagonistic interaction with dopamine receptors are consistent with a key role for A_{2A} receptors in motor activity control (Ferré, Fuxe, von Euler, Johansson, & Fredholm, 1992, 1997; Fuxe, Ferré, Zoli, & Agnati, 1998). Previous work on mice lacking A_{2A} receptors (Chen et al., 1999; Ledent et al., 1997) indicates an involvement of these receptors in neurorepair mechanisms, anxiety and aggressive behaviours. In those mice, the lack of A_{2A} receptors also resulted in reduced startle habituation and prepulse inhibition (Wang, Short, Ledent, Lawrence, & van den Buuse, 2003) which are impairments in sensorimotor gating also seen in schizophrenia (Braff et al., 1978) and after treatment of rats with dopamine receptor agonists (Swerdlow et al., 1995). Pharmacological studies have indicated that combined A_1–A_{2A} receptor blockade exerts facilitative effects on spatial memory performance in rats, and both receptor subtypes might also be involved in hippocampal long-term potentiation (Arai, Kessler, & Lynch, 1990; Kessey, Trommer, Overstreet, Ji, & Mogul, 1997; Rebola et al., 2003). Furthermore, there is evidence for the involvement of A_{2A} receptors in striatal long-term potentiation (d'Alcantara, Ledent, Swillens, & Schiffmann, 2001). However, the specific role of A_{2A} receptors in learning and memory is still unclear, with A_1 receptors apparently having a more dominant role (Hauber & Bareiss, 2001; Moreau & Huber, 1999; Suzuki et al., 1993).

Short-term or 'working' memory is a cognitive process relevant for keeping track of important information and ideas that becomes critical for human reasoning and judgement. Working memory depends on the integrity of prefrontal function although hippocampus, inferior parietal cortex, caudate nucleus and dorsomedial nucleus of the thalamus are brain areas also known to play a relevant role in its neural circuitry (reviewed by Castner, Goldman-Rakic, & Williams, 2004). At the neuropsychiatric level, working memory is the core and most consistently observed cognitive deficit exhibited by patients with schizophrenia (Park & Holzman, 1992). Prefrontal dysfunction linked to altered dopaminergic and glutamatergic transmission or to disruption of mesencephalocortical pathways is also suggested to cause working memory deficits in Parkinson's disease patients (García-Munoz, Young, & Groves, 1991). Studies in both human and subhuman primates have also identified the prefrontal cortex as a key node in the functional neural networks of working memory and its deficits (Goldman-Rakic, 1994). In rodents, experimentally induced models of prefrontal dysfunction including amphetamine sensitisation, subchronic phencyclidine and neurodevelopmental insults exhibit spatial and object working memory deficits in delayed response procedures or within-day learning in several kind of mazes (Morris water maze, T-maze, Y-maze, Radial maze, etc), and social and object recognition tests. where working memory can be distinguished from long-term or 'reference' memory associated with day-to-day learning (reviewed by Castner et al., 2004).

It is of substantial interest that stimulation of A_{2A} receptors, like dopamine D_1 receptors, increases cAMP signalling and neuronal excitability, especially in view of the fact that not only reductions but also increases in D_1 receptor-mediated dopaminergic transmission lead to working memory deficits (Goldman-Rakic, Muly, & Williams, 2000). Therefore, in order to study the consequences of increased central A_{2A} receptor signalling, we established a novel transgenic rat model TGR(NSEhA2A) overexpressing this receptor. Preferential overexpression of A_{2A} receptors was located in some of these neuroanatomical substrates, where also dysregulation of dopamine signalling is associated with working memory impairments and schizophrenia. Thereafter, we submitted the animals to a behavioural battery to ascertain motor activity, anxiety, and learning–memory functions. The results revealed a marked neuronal A_{2A} overexpression especially in cortical regions that may be critical for the working memory deficits observed. In view of the existence of A_{2A}/D_2 and $A_{2A}/mGlu_5$ heteromeric complexes in the striatum (Canals et al., 2003; Ferré et al., 2002; Fuxe et al., 2005; Hillion et al., 2002) also D_2 and $mGlu_5$ proteins were analysed in immunoblotting experiments on the striatum of the TGR(NSEhA2A) overexpressing the A_{2A} receptors to further understand the neurobiological basis for the working memory deficits observed.

2. Methods and materials

2.1. Generation of transgenic animals

Transgenic rats for the overexpression of A_{2A} receptors in the central nervous system were generated by microinjection of a DNA construct into the male pronucleus of Sprague–Dawley rat zygotes with established methods (Popova, Krivokharchenko, Ganten, & Bader, 2002). The construct contained a full-length human A_{2A} cDNA cloned into an expression vector 3′ of the 1.8 kb rat neuron-specific enolase (NSE) promoter and 5′ of an intron/polyadenylation cassette of SV40 virus (see Fig. 1A).

2.2. Genotyping of rats

Transgenic rats were identified by PCR (30 cycles, 54 °C annealing temperature) on their genomic DNA isolated from tail biopsies by the use of the following transgene-specific primers: SV40ipa5: 5′-GAAGGAACC TTACTTCTGTGG-3′ and SV40ipa3: 5′-TCTTGTATAGCAGTGCAG C-3′ (see Fig. 1A).

2.3. RNase protection assay

Total RNA was isolated from rat tissues by TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. With this RNA, transgene expression was measured by RNase protection assay (RPA) as described previously (Silva et al., 2000) using a commercially available kit (RPAII, Ambion) and a probe covering the 5′-terminal 125 nucleotides of the transgenic mRNA (see Fig. 1A).