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The usefulness of the spontaneously hypertensive rat to model attention-deficit/hyperactivity disorder (ADHD) may be explained by the differential expression of dopamine-related genes in the brain

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Abstract

Spontaneously hypertensive rats (SHR) are considered to represent a genetic animal model for attention-deficit hyperactivity disorder (ADHD). In the present studies, we compared the locomotor activity, learning and memory functions of juvenile male SHR, with age- and gender-matched genetic control Wistar–Kyoto rats (WKY). In addition, we investigated potential differences in brain morphology by magnetic resonance imaging (MRI). In other complimentary studies of the central nervous system, we used real-time PCR to examine the levels of several dopaminergic-related genes, including those coding for the five major subtypes of dopamine receptor (D1, D2, D3, D4 and D5), those coding for enzymes responsible for synthesizing tyrosine hydroxylase and dopamine-β-hydroxylase, and those coding for the dopamine transporter. Our data revealed that SHR were more active than WKY in the open field (OF) test. Also, SHR appeared less attentive, exhibiting inhibition deficit, but in the absence of memory deficits relative to spatial learning. The MRI studies revealed that SHR had a significantly smaller vermis cerebelli and caudate–putamen (CPu), and there was also a significantly lower level of dopamine D4 receptor gene expression and protein synthesis in the prefrontal cortex (PFC) of SHR. However, there were no significant differences between the expression of other dopaminergic-related genes in the midbrain, prefrontal cortex, temporal cortex, striatum, or amygdala of SHR and WKY. The data are similar to the situation seen in ADHD patients, relative to normal volunteers, and it is possible that the hypo-dopaminergic state involves a down regulation of dopamine D4 receptors, rather than a general down-regulation of catecholamine synthesis. In conclusion, the molecular and behavioural data that we obtained provide new information that may be relevant to understanding ADHD in man.

Keywords: Spontaneously hypertensive rat (SHR); Open field test (OF test); Morris water maze; Prepulse inhibition; Water finding; Magnetic resonance imaging (MRI); Real-time PCR

1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is characterized by age-inappropriate inattention, impulsiveness, and hyperactivity (Wilens et al., 2002). Approximately 5–10% of school-aged children worldwide have ADHD, with the incidence being three times higher in boys than girls (Barkley and Biederman, 1997; Brown et al., 2001; Hinshaw, 1992; Scahill and Schwab-Stone, 2000). Unfortunately, the disease continues into adulthood in 30–70% of patients (Silver, 2000). It has been proposed that ADHD may lead to memory deficits, delinquency, substance abuse, and problematic personality disorders, in addition to constituting one of the highest risk factors for other mental illnesses (Taylor, 1998). Although the precise etiology and pathological mechanisms underlying ADHD are poorly understood, accumulating data indicates that genetics may influence its incidence. For example, studies focusing on twins showed that the heritability of ADHD was 0.80, indicating a strong genetic predisposition (Faraone and Biederman, 1998). Other case-controlled and/or family-based

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association studies have targeted several specific candidate genes. This approach has identified the potential involvement of dopamine D2, D4 and D5 receptor genes, and serotonin 2A genes, as well as the genes coding the dopamine and serotonin transporters (LaHoste et al., 1996). Indeed, the dopamine D4 receptor gene has been one of the most studied genes in ADHD, with a recent quantitative trait study showing that a 7-repeat allele may be linked to specific neuropsychological behaviours to give rise to a new phenotype of ADHD (Swanson et al., 1998).

In addition to the molecular studies, magnetic resonance imaging (MRI) of the brains of ADHD patients has revealed a smaller sized basal ganglia, corpus callosum, prefrontal cortex (PFC), and cerebellum compared to normal individuals (Castellanos et al., 1996; Filipek et al., 1997). These observations link with the molecular findings, since dopaminergic projections from midbrain ventral tegmental area (VTA) to the striatal and prefrontal cortical areas, play a major role in motor control, and attention and impulsion (Eells, 2003).

Adult spontaneously hypertensive rats (SHR) are commonly used in cardiovascular research. However, juvenile (4–6-week old) SHR are being used increasingly to model ADHD because they are hyperactive (Castanon et al., 1993; Sagvolden et al., 1992; Whitehorn et al., 1983), inattentive (Berger and Sagvolden, 1998) and impulsive (Boix et al., 1998). These three behavioral characteristics correlate with the classical symptoms exhibited by children with ADHD (Hendley, 2000; Sagvolden, 2000), and are seen prior to the development of hypertension in these animals (Sagvolden et al., 2005b). Notwithstanding these important behavioral correlates, SHR also have a lower turnover of dopamine in the VTA, striatum, and frontal cortex that is relevant to the clinical situation (de Villiers et al., 1995; Linthorst et al., 1994).

The level and function of particular transmitter in the neuroeffector junction is controlled by a variety of factors. In the case of dopamine, this includes the level of activity of tyrosine hydroxyls (TH), which catalyzes the conversion of tyrosine to dihydroxyphenylalanine (DOPA); dopa decarboxylase (DDC) which catalyzes the conversion of DOPA to dopamine; dopamine-β-hydroxylase (DβH) which catalyzes the formation of noradrenaline from dopamine and there are also metabolic enzymes that can deactivate dopamine, including monoamine oxidase.

Dopamine itself interacts with five major dopamine receptors and is removed from the synaptic cleft by a specific dopamine transporter (Missale et al., 1998). However, no studies have investigated the possibility that genes coding the synthetic/metabolic pathways of dopamine, or its receptors, are differentially expressed in SHR compared to its genetic control. In the present studies, therefore, we decided to compare the locomotors activity, attention, and learning and memory functions of juvenile male SHR with age- and gender-matched genetic control Wistar–Kyoto rats (WKY), with particular emphasis on the potential differences of gene expression relevant to the dopaminergic system in different brain areas. MRI was also employed to probe for potential morphological differences between brain areas of SHR and WKY. It was hoped that the results of the study would help address issues relating to the appropriateness of the use of juvenile SHR to model ADHD.

2. Experimental procedures

2.1. Animals

Juvenile male SHR aged 4–6-week old, and age- and gender-matched genetic control WKY, were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong (CUHK). The original colony originated from Harlan Olac, UK. The rats had free access to standard laboratory rodent chow and water and were housed in a room with 12 h light–dark cycle; temperature and humidity were maintained at 22 ± 1 °C and 45–55%, respectively. The experiments were approved by the Animal Experimentation Ethics Committee, CUHK.

2.2. Behavioral assessments

2.2.1. Open field test

The open field (OF) box was a 50 cm × 50 cm × 50 cm cube constructed from black Plexiglas. SHR ($n = 15$) and WKY ($n = 15$) were placed into the box and were videotaped for 5 min using an Animal Behavior Recognition System (Institute of Psychology, Chinese Academy of Sciences, Beijing, China). The total distance travelled in 5 min was recorded and used to define the basal spontaneous locomotor activity of the animals (Ferguson et al., 1993). Urine and faeces were removed from the box between test sessions. The rats were allowed to three sessions spaced 24 h apart to habituate to the boxes prior to testing. This was done to reduce the potential anxiety caused by the novel environment. Each session was 20 min long.

2.2.2. Morris water maze

2.2.2.1. Experiment 1: special learning and reference memory. The water maze was constructed from black polypropylene (120 cm diameter). It had a diameter of 10 cm platform that could be submerged 1 cm below the water surface. The water in the pool was maintained at $23\ ^{\circ}C \pm 1\ ^{\circ}C$. The pool was divided into four quadrants designated Northeast (NE), Northwest (NW), Southeast (SE) and Southwest (SW). In cue training sessions, four cardboard cue cards, having different colors and shapes were placed 25 cm above the water level and at each quadrant of the platform. Video of an animal's performance was recorded by a camera situated on the ceiling, directly above the center of the pool. The videotape records were analyzed using a custom made tracking system (Department of Biochemistry, CUHK).

Rats ($n = 10$ per line) received four trials in the water maze on each of the 4 days (Terry et al., 2000). During testing, the submerged platform remained stationary in one quadrant of the maze, and the latency to find the platform was recorded. Each trial consisted of an individual rat being placed carefully into the water, facing the outer edge of the pool, at one of the three possible starting points, excluding the point of the platform. The starting location for each trial was determined randomly. A trail was complete when the rat reached the platform and remained on it for 20 s. The latency to find the platform was recorded. If the rat did not reach the platform within 90 s, the trial was terminated, and the rat was subsequently placed on the platform for 20 s. Rats were then transferred to a dry holding cage until the next trial. On the 5th day, rats received an additional 90 s probe trial, in which a platform was not present in the pool. Rats were placed in the pool, as before, and the average distance from the target was recorded.

2.2.2.2. Experiment 2: influence of an interferent stimulus on cued Morris water maze. We have used an interferent stimulus to provide basal data on attention in SHR (Anisman and McIntyre, 2002). In these studies, we used rats after being trained in experiment 1. In experiment 1, SHR and WKY ($n = 10$) received 4 days of training (four trials per day) in the cued Morris water maze task as described earlier. On the 5th day, rats received an additional 90 s probe trial. Then on the 6th day, start of experiment 2, a second above-water cue was present, but it was irrelevant to the submerged platform position (i.e. the