α₂-Adrenoceptor Mediated Inhibition of [³H]Dopamine Release from Nucleus Accumbens Slices and Monoamine Levels in a Rat Model for Attention-Deficit Hyperactivity Disorder

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The spontaneously hypertensive rat (SHR) has been proposed as an animal model for attention-deficit hyperactivity disorder (ADHD). The behavioural problems have been suggested to be secondary to altered reinforcement mechanisms in which nucleus accumbens dopaminergic activity plays an important role. Interaction between the noradrenergic and dopaminergic system in the nucleus accumbens has been implicated in the locomotor hyperactivity and impaired discriminative performance of SHR. The present study therefore investigated whether there was any change in the α₂-adrenoceptor mediated inhibition of dopamine release from nucleus accumbens slices of SHR in comparison with their normotensive Wistar-Kyoto (WKY) controls. The electrically stimulated release of [³H]dopamine (DA) from nucleus accumbens slices was decreased to a similar extent by UK14,304, an α₂-adrenoceptor agonist, in SHR and WKY. Basal norepinephrine (NE) levels were increased in locus coeruleus (LC) and A₂ noradrenergic nuclei, but not in the A₁ nucleus of SHR, while basal serotonin (5-HT) levels were increased in all these pons-medulla nuclei. These results suggest that a primarily dysfunctional LC and A₂ nucleus does not have a secondary effect on dopaminergic transmission in the nucleus accumbens via α₂-adrenoceptor mediated inhibition of DA release. Basal monoamine levels in several brain areas of SHR were significantly different from that of WKY. DA, and 5-HT turnover were decreased in SHR versus WKY suggesting hypofunctional dopaminergic and serotonergic systems in some brain areas of SHR.

KEY WORDS: SHR; ADHD; reinforcement; nucleus accumbens; α₂-adrenoceptor; dopamine release; monoamines.

INTRODUCTION

According to the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (APA DSM IIIR), children with attention-deficit hyperactivity disorder (ADHD) are characterized by inappropriate degrees of inattention, impulsiveness and hyperactivity. Furthermore, these children have a reduced tolerance for delay of gratification (1). It is generally agreed that 2–6% of all school-age children suffer from ADHD (2) and that it has a genetic component (3). It has been repeatedly proposed that spontaneously hypertensive rats (SHR) may be useful as a genetic animal model of ADHD (4–6). The SHR model shows the major behavioural characteristics of ADHD, namely hyperactivity and discrimination problems as well as altered

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reactivity to psychomotor stimulants (6), the drugs of choice in the treatment of ADHD. It has been suggested that altered reinforcement mechanisms may underlie these behavioural changes in SHR (4–6). Animal experiments indicate that the nucleus accumbens dopaminergic activity plays an important role in reinforcement (7). Thus, altered dopaminergic function may be important for the understanding of the altered behaviour both in SHR as well as ADHD. In fact, dopaminergic function has been reported to be increased (8), decreased (9) or unchanged (10) in the striatum of SHR when compared to the progenitor normotensive Wistar-Kyoto rat (WKY). Reduced dopamine (DA) turnover has also been found in the medulla oblongata and frontal cortex of SHR (11).

The nucleus accumbens receives considerable noradrenergic input (12–14). This input inhibits DA release via α2-adrenoceptors (15,16). Thus, it is possible that primary changes in the noradrenergic system may have secondary effects on the dopaminergic system in the nucleus accumbens of SHR. Such strain differences in noradrenergic characteristics are of clinical relevance as altered functions of NE may be involved in the genesis of ADHD (4,17).

It has also been suggested that the hyperactivity of SHR is related to noradrenergic dysfunction in the CNS (18). However, the data from the available studies with SHR are contradictory. Measurements of enzyme activities, amine content or rates of amine disappearance following pharmacological inhibition of amine synthesis suggested augmented (19–22), attenuated (23,24) or unchanged function (8,9,11) of noradrenergic neurons in the brainstem and other areas of SHR brain. Desensitization of α2-adrenoceptors in the brain of SHR has also been demonstrated (25).

The aim of the present study was to investigate whether there were any changes in the activity of the NE neurons which innervate the nucleus accumbens and whether this could lead to a change in the α2-adrenoceptor mediated inhibition of DA release in the nucleus accumbens of SHR. This was accomplished by measuring monoamine and metabolite levels in several brain areas and by studying the effect of a selective α2-adrenoceptor agonist, UK14,304 (26), on the electrically stimulated release of [3H]DA from nucleus accumbens slices of SHR and WKY, using an in vitro superfusion technique.

**EXPERIMENTAL PROCEDURE**

**Animals.** Male SHR and WKY (245–300 g, 11–13 weeks old, bred from SHR and WKY obtained from the University of the Witwatersrand) were used in this study. Animals were weaned at the age of 4 weeks and housed in groups of 5 rats per cage under standard laboratory conditions (12 h light-dark cycle, free access to rat food and water). Rats were transported to the laboratory at least one hour before sacrifice. After decapitation the brains were rapidly removed and placed on ice. The nucleus accumbens was dissected (27) and sliced (0.3 × 0.3 mm) with a McIlwain tissue chopper prior to use in superfusion studies.

**Monoamine Determination.** The remaining caudal part of the brains was immediately mounted on its caudal base on a chuck in O.C.T. embedding medium and frozen in liquid nitrogen. The ventral tegmental area (VTA), substantia nigra and the noradrenergic nuclei, locus coeruleus (LC), A1, and A2, were removed according the micro-punch methods of Palkovits and Brownstein (28). Tissues were stored at −100°C until analysis of NE, DA, serotonin (5-HT) and metabolites by high-performance liquid chromatography with electrochemical detection (14). Briefly, brain tissues were sonicated in 0.1 M perchloric acid containing 0.3 mM Na2EDTA and 0.5 mM sodium metabisulphite. Following centrifugation at 4°C (21,000 g, 15 min) ascorbic acid oxidase was added to the supernatant which enabled the NE peak to be well separated from the solvent front, a large proportion of which is ascorbic acid. The supernatants were filtered through 0.2 μm microfilters and 20 μl of filtrate were injected onto a 250 × 4.6 mm Bio-phase ODS column (particle size 5 μm). A glcassy glassy carbon electrode set at +0.70 V vs the Ag/AgCl reference electrode and a sensitivity of 2 nA/V were used. The mobile phase comprised 0.1 M formate buffer (pH 3.85), 0.5 mM EDTA, 5 mM sodium heptane sulphonic acid, 6% v/v methanol and 4% v/v acetonitrile. Results were expressed as ng/g wet weight of tissue.

**Superfusion Studies.** Nucleus accumbens slices were suspended in 50 volumes of aerated Krebs buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl2·6H2O, 1.0 mM NaH2PO4, 1.3 mM CaCl2, 25 mM NaHCO3, 11 mM glucose and 0.04 mM EDTA), adjusted to pH 7.3 by aeration with 95%O2/5%CO2. The slices were incubated in Krebs buffer containing an anti-oxidant, ascorbate (1 mg/ml), a MAO inhibitor, pargyline (10 μM), a NE uptake inhibitor, desipramine (29, 1 μM) and a 5-HT uptake inhibitor, citalopram (30, 1 μM). After a 10 min equilibration period, [3H]DA was added to the tissue slice preparation at 37°C to give a final concentration of 0.04 μM [3H]DA (40 Ci/mmol). After an additional 15 min incubation period, slices were washed with ice cold incubation buffer, transferred to superfusion chambers (8 in total) and continuously perfused at 37°C with Krebs buffer (0.25 ml/min) as previously described (27). Collection of 5 min fractions began after a 75 min wash period with drug-free Krebs buffer. The slices were stimulated for 2 min using biphasic square wave pulses (2 ms duration, 16 mA amplitude and 5 Hz frequency) after collection of two fractions of eluate had commenced ([H]DA). Collection of 5 min fractions began after a 75 min wash period with drug-free Krebs buffer. The slices were stimulated for 2 min using biphasic square wave pulses (2 ms duration, 16 mA amplitude and 5 Hz frequency) after collection of two fractions of eluate had commenced ([H]DA). A selective α2-adrenoceptor agonist, UK14,304 (1 μM), was added to the test chambers after a further six fractions had been collected, i.e., two fractions before the second stimulation, S2, was applied. The drug remained present in the superfusion buffer while a further five fractions were collected. The radioactivity in the eluate fractions as well as the residual radioactivity in the tissue slices at the end of the experiment was determined in a Packard 2200 CA TRI-CARB liquid scintillation analyzer.

In order to correct for differences in the amount of tissue in each superfusion chamber, the data were expressed as fractional release, which is the amount of radioactivity released in each fraction divided by the total amount of radioactivity present in the tissue at the time of release. The area of the peak of fractional release of radioactivity (S1 and S2) was used as a measure of neurotransmitter release. S1 represents the electrically stimulated release of transmitter before