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Autoradiographic and pharmacological studies on the role of dopamine D3 receptors in genetically dystonic (dt^{sz}) hamsters

Svenja E. Sander [a], Roger Raymond [b], José N. Nobrega [b], Angelika Richter [a,*]

[a] Institute of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Freie Universität Berlin, Koserstr. 20, 14195 Berlin, Germany
[b] Neuroimaging Research Section, Centre for Addiction and Mental Health, Toronto, Ontario, Canada M5T 1R8

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ABSTRACT

Previous examinations demonstrated periodic increases in striatal extracellular dopamine levels during dystonic attacks and changes in dopamine D1 and D2 receptor binding in the dt^{sz} mutant hamster, an animal model of paroxysmal non-kinesiogenic dyskinesia in which dystonic episodes can be induced by stress. Since dopamine D3 receptors are involved in the regulation of striatal dopamine release, D3 receptor function was investigated by autoradiographic and pharmacological examinations in mutant hamsters in the present study. [^{125}I]7-[[(E)-3-iodoprop-2-enyl]-propylamino]-5,6,7,8-tetrahydronaphthalen-2-ol ([^{125}I]7-OH-PIPAT) binding was not significantly altered in the striatum, n. accumbens, ventral pallidum or cerebellum in dt^{sz} hamsters in comparison to non-dystonic control hamsters. In line with the unaltered D3 receptor binding, the preferential dopamine D3 versus D2 receptor antagonist U-99194 (5,6-dimethoxy-*N*,*N*-dipropyl-2,3-dihydro-1H-inden-2-amine hydrochloride) did not exert significant effects on the severity of dystonia in dt^{sz} hamsters at doses of 10 to 40 mg/kg which induced hyperlocomotion. These results suggest that periodic elevations of dopamine levels in these animals are not related to D3 receptor dysfunctions.

1. Introduction

Dystonia is a syndrome of involuntary muscle contractions causing twisting movements and abnormal postures. This common movement disorder shows a heterogeneous etiology and a wide clinical spectrum (Breakefield et al., 2008; Fahn et al., 1998). The pathophysiology is not well understood, but changes in striatal dopaminergic transmission have been suggested to be involved in various types of dystonias and in dystonia-associated dyskinesias (Breakefield et al., 2008; Wichmann, 2008). In the dt^{sz} mutant hamster, an animal model of paroxysmal dystonia (Richter, 2005), previous studies have shown abnormalities in dopaminergic transmission, such as episodic increases in extracellular dopamine levels in the dorsal striatum during the manifestation of dystonic attacks and regional changes in dopamine D1 and D2 receptor binding (Hamann and Richter, 2004; Nobrega et al., 1996; Rehders et al., 2000). However, the role of dopamine D3 receptors in primary dystonias, both in patients and in animal models, is unknown.

Among the five subtypes of dopamine receptors (D1–D5), D1 and D5 show a D1-like pharmacological profile, while D2, D3, and D4 receptors have a D2-like pharmacology. Dopamine has a 70-fold higher affinity for the dopamine D3 receptor than for D1 or D2 receptors. Post- and presynaptic dopamine D3 receptors are particularly expressed in brain regions which regulate limbic and motor functions (Joyce, 2001; Schwartz et al., 1993, 2000). In the human striatum, D2 receptors are expressed in the dorsal striatum and the nucleus accumbens, while D3 receptors are primarily localized in the accumbens and the ventral side of the putamen (Meador-Woodruff et al., 1996).

Interestingly, levodopa-induced dyskinesias were found to be accompanied by enhanced expression of dopamine D3 receptors on striatonigral neurons of the dorsal striatum in a rat model (Schwartz et al., 1998). This observation indicates that D3 receptors deserve attention in dystonias, because levodopa-induced dyskinesias can be associated with dystonic symptoms. Mice with a genetic deletion of the dopamine D3 receptor exhibit hyperlocomotion. In these mice, significantly elevated extracellular dopamine levels in the striatum (Joseph et al., 2002) suggest an important role of the D3 receptor in the regulation of dopaminergic activity. The dopamine D3 autoreceptor seems to control the phasic, but not tonic activity of dopamine neurons (Sokoloff et al., 2006). Thus, the previous finding of periodic elevations of striatal dopamine levels in dystonic hamsters might be related to altered dopamine D3 receptor function.

In the present study we therefore examined the pathophysiological role of dopamine D3 receptors in dystonia by autoradiographic analyses in the dt^{sz} mutant. In addition, the effects of the preferential dopamine D3 versus D2 receptor antagonist 5,6-dimethoxy-N,N-dipropyl-2,3-dihydro-1H-inden-2-amine hydrochloride (U-99194) on the severity of dystonia were examined. The high degree of homology between the binding sites of the D2 and D3 dopamine

* Corresponding author. Tel.: +49 30 83854317; fax: +49 30 83853112.
E-mail address: richtera@zedat.fu-berlin.de (A. Richter).

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receptor subtypes hampered the development of D3 receptor subtype selective compounds. Systemically active and highly selective dopamine D3 receptor agonists and antagonists are not available, but U-99194 exhibits a 30-fold preference for the D3 receptor compared to the D2 receptors and has a 60-fold preference for D3 than for D4 receptors (Audinot et al., 1998; Hackling and Stark, 2002).

2. Material and methods

2.1. Animals

The study was carried out in groups of male and female dt^{sz} mutant Syrian golden hamsters (inbred line) and age- and sex-matched non-dystonic control hamsters, which were obtained by selective breeding as described previously (Richter and Löscher, 1998). All dystonic and control hamsters were born and kept under the same controlled environmental conditions. All experiments were done in compliance with the German Animal Welfare Act (G0160/05).

2.2. Severity-score of dystonia and induction of dystonic episodes

As reported previously in detail, motor impairments in dt^{sz} hamsters are transmitted by a recessive gene and show several features in common with human primary paroxysmal non-kinesiogenic dystonia (paroxysmal dystonic choreoathetosis), characterized by long-lasting dystonic attacks (Richter, 2005). In mutant hamsters, dystonic attacks can be reproducibly induced by a triple stimulation technique (Richter and Löscher, 1998), i.e., stressful stimuli consisting of (1) taking the animal from its home cage and placing it on a balance, (2) intraperitoneal (i.p.) injection of saline (or of U-99194, see below), and (3) placement of the animal in a new plastic cage. After this procedure, dt^{sz} hamsters develop a sequence of abnormal movements and postures. Therefore, the severity of dystonia can be rated by the following score system (Richter and Löscher, 1998): stage 1, flat body posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with hyperextended forepaws; stage 3, hyperextended hindlimbs so that the animals appear to walk on tiptoes; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, immobilisation in a twisted, hunched posture with hind- and forelimbs tonically extended forward. After reaching the individual maximum stage the hamsters recover within 2–5 h. The individual maximum stage of dystonia is usually reached within 3 h after the hamsters were placed in the new cage. Therefore, the animals have to be observed for 3 h after the induction of dystonic attacks to determine the individual maximum stage reached after administration of drugs or of vehicle (for pre- and post-drug control recordings).

In the present study, all animals were examined for the presence of dystonia after weaning at the age of 21 days by the triple stimulation procedure, including injections of saline. All hamsters used for investigations were repeatedly tested by triple stimulations (injections of saline) every 2 to 3 days after weaning until the severity of dystonia and latencies to the different stages were reproducible.

2.3. Autoradiographic analyses

Quantitative autoradiographic analysis of dopamine D3 receptors was carried out groups of 7 mutant hamsters and 8 age- and gender-matched non-dystonic control hamsters. The hamsters were killed by decapitation at an age of 35 days and the brains were rapidly removed, frozen on dry ice, and then stored at −80 °C until cryostat sectioning. Coronal cryostat sections (20 µm) from frozen brains were cut at −20 °C (Leica Microsystems, Wetzlar, Germany), thaw-mounted onto Superfrost slides (Fisher Scientific, Ottawa, ON, Canada) and then stored at −80 °C until binding assays were performed. As recently described (Harrison and Nobrega, 2009), for D3 receptor autoradiography, slides were pre-incubated for 30 min at 30 °C in 50 mM TRIS buffer (pH 7.4), containing 100 mM NaCl, and 50 mM guanylyl-imido-diphosphate. For total binding, slides were incubated for 90 min at room temperature in a 50-mM TRIS buffer (pH 7.0) containing 40 mM NaCl, 50 mM guanylyl-imido-diphosphate (Sigma-Aldrich, Canada), 5 mM 1,3-di(2-tolyl)guanidine (Sigma-Aldrich), 1 mM ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich), and 0.2 nM [^{125}I]7-OH-PIPAT (PerkinElmer Life Sciences, Boston, MA, USA). For nonspecific binding, representative slides were incubated with 10 mM unlabeled 7-OH-DPAT HBr (Tocris Bioscience, Ellisville, MO, USA) added to the radioligand solution. Slides were washed three times in 50 mM TRIS buffer (pH 7.4) at 4 °C for 30 min each then dipped in ice-cold distilled water for 10 s and dried under a steady stream of cool air. Dried slides along with calibrated standards were exposed on Kodak Biomax MR-1 film for 3 days and then developed. Densitometric analysis was performed using the MCID software program (Imaging Research, St. Catherine's, ON, Canada). A standard curve was generated that relates optical density to known quantities of [^{125}I] (in microcurie per gram of tissue). For any subject, the final binding value for any given brain region represented an average of multiple readings on 3–6 brain sections. Brain regions were defined according to Stereotaxic Atlas of Hamster Brain (Morin and Wood, 2001). Binding values were averaged for each region and each subject, and group means were compared independently for each of the regions sampled. Data were analysed by ANOVA using Systat 5.0 Software (Systat, Evanston, IL), followed by independent t Test comparisons for brain regions where significant F values (p at least <0.05) were obtained.

2.4. Pharmacological studies

The preferential dopamine D3 versus D2 receptor antagonist 2,3-dihydro-5,6-dimethoxy-*N*,*N*-dipropyl-1*H*-inden-2-amine-maleate (U-99194) (Tocris Cookson, Avonmouth, UK) was freshly dissolved in saline prior to the experiments. The effects of 10, 20 and 40 mg/kg i.p. were examined in groups of 7–10 dt^{sz} hamsters at an age between 30 and 45 days, when the animals are highly sensitive to stressful stimuli and develop high severity scores. The doses were chosen on the basis of several previously described experiments in rats and mice (Gyertyán and Sághy, 2004; Jones et al., 2007).

Dystonic attacks were induced by the procedure of triple stimulation, as described above, but instead of saline the active compound was injected (injection volume: 5 ml/kg i.p.). Pre- and post-drug control trials with the vehicle (injection volume: 5 ml/kg isotonic saline i.p.) were undertaken 2–3 days before and 2–3 days after drug testing in the same animals. Since the individual maximum stage of dystonia is usually reached within 3 h, the hamsters were observed for 3 h after triple stimulation. During this period, the severity of dystonia, the latencies to the different stages and the side effects were noted. The side effects were not quantified, but locomotor activity was determined according to a score system, as previously described (Richter and Löscher, 1995).

The significance of differences in severity of dystonia and in latency to the onset of dystonia (latency to occurrence of unequivocal dystonic symptoms, stage 2) between control trials (pre- and post-drug) and drug trial in the same group of animals was calculated by the Friedman test. If there was a significant difference (at least $p<0.05$), the Wilcoxon signed rank test for paired replicates was used post hoc to determine which pairs differed. Significant differences in the locomotor activity scores between different doses were determined by the Mann–Whitney Rank Sum Test.

3. Results

The distribution of dopamine D3 receptors in hamster brains (Fig. 1) was comparable to the previously reported [^{125}I]-7-OH-PIPAT