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Effects of intrastriatal injections of glutamate receptor antagonists on the severity of paroxysmal dystonia in the dt^{sz} mutant

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Abstract

Imbalances of the glutamatergic system are implicated in the pathophysiology of various basal ganglia disorders, but few is known about their role in dystonia, a common neurological syndrome in which involuntary muscle co-contractions lead to twisting movements and abnormal postures. Previous systemic administrations of glutamate receptor antagonists in dt^{sz} hamsters, an animal model of primary paroxysmal dystonia, exerted antidystonic effects and electrophysiological experiments pointed to an enhanced corticostriatal glutamatergic activity. In order to examine the pathophysiological relevance of these findings, we performed striatal microinjections of the α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA) receptor antagonist 2,3-dioxo-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) and the *N*-methyl-D-aspartate (NMDA) receptor antagonists D(−)-2-amino-5-phosphopentanoic acid (AP-5), (*R*)-(+)-3-amino-1-hydroxypyrrolidin-2-one (HA-966) and dizocilpine (MK-801). The striatal application of NBQX reduced the severity and increased the latency to onset of dystonia significantly only at a dosage of 0.08 μg per hemisphere, lower (0.03 μg) and higher dosages (0.16 μg and 0.32 μg) failed to exert comparable effects on the severity. None of the striatal injected NMDA receptor antagonists influenced the severity of the dystonic attacks in the mutant hamster. The combined application of NBQX (0.08 μg) with AP-5 (1.0 μg) failed to exert synergistic antidystonic effects, but the beneficial effect on the severity of dystonia of the single application of NBQX was reproduced. Therefore, corticostriatal glutamatergic overactivity mediated by AMPA receptors, but not by NMDA receptors, is possibly important for the manifestation of dystonic attacks in the dt^{sz} hamster mutant.

Keywords: Basal ganglia; Dyskinesia; Glutamate; Movement disorder; Striatum

1. Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) and is thought to be involved in the pathophysiology of various CNS disorders. Beside the three types of ionotropic receptors, namely α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors, glutamate activates several types of metabotropic receptors (Parsons et al., 1998). Today, the widest knowledge exists about NMDA and AMPA receptors and their implication in diseases.

Ionotropic glutamate receptors are located on various neurons of the basal ganglia nuclei, such as striatal projection neurons of the direct and indirect pathway and striatal interneurons. Therefore, imbalances of the glutamatergic system are hypothesized to contribute to several basal ganglia disorders, like Parkinson's disease, Chorea Huntington and dyskinesias (Kulkarni and Naidu, 2001; Li et al., 2004). In dystonia, a common movement disorder in which prolonged muscle co-contractions cause sustained twisting movements and abnormal postures (Fahn et al., 1998), the pathophysiology is still unclear, which hampers the development of rational therapies. There is evidence for a reduced activity of inhibitory neurons of the globus pallidus externus in dystonic patients (Vitek, 2002) and in the entopeduncular nucleus (the homolog of the globus pallidus externus in rodents) of the dt^{sz} mutant hamster (Gernert et al., 2000). This is possibly related to an overactivity of striatal inhibitory neurons, which project to this basal ganglia output structure.

Animal models for the different types of dystonia are rare, and only a few of them are well characterized. The dt^{sz} mutant

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hamster shares numerous phenotypical characteristics with the primary paroxysmal non-kinesiogenic dystonic choreoathetosis (briefly: paroxysmal dystonia) in patients, such as the clinical symptoms and the responsiveness to drugs. In this disorder, periods of generalized dystonia (the predominant symptom) can be induced by stress and caffeine. They can appear several times daily and last from 5 min up to 4 h (Nardocci et al., 2002; Richter and Löscher, 1998). The symptoms of primary paroxysmal dystonia occur in the absence of any lesions that can be defined by either standard post mortem pathological investigations or in vivo imaging (Nardocci et al., 2002).

Whereas clinical trails concerning the pathophysiological role of glutamate in dystonic patients are barely existent and hard to interpret (Richter and Löscher, 1998), there is evidence that a glutamatergic overactivity contributes to the dystonic attacks in the dt^{sz} hamster mutant. Systemic administrations of different glutamate receptor antagonists proved to have beneficial effects on dystonia in dt^{sz} hamsters (Löscher and Richter, 1993; Richter et al., 1991, 1993), but the relevant brain structures for this drug action are still unclear. A pathophysiological relevance for a nigrostriatal dopaminergic overactivity has already been shown by previous microdialysis studies of extracellular dopamine levels and striatal microinjections of dopamine receptor antagonists (Hamann and Richter, 2004; Rehders et al., 2000). The dt^{sz} hamster has an ontogenetic deficit of striatal GABAergic interneurons (Gernert et al., 2000; Hamann et al., 2005; Sander et al., 2006), which are the major inhibitory source within the striatum. As presumed in the case of the dopaminergic overactivity, a GABAergic disinhibition may result in corticostriatal glutamatergic overactivity which may lead by activation of GABAergic projection neurons to a decreased activity of the basal ganglia output structure. The resultant disinhibition of the thalamus is thought to contribute to the manifestation of a dystonic episode.

Recent electrophysiological studies, including field responses, paired-pulse accentuation and LTP, suggest an involvement of the corticostriatal pathway in the pathophysiology of dystonic attacks in dt^{sz} hamsters, as they indicated an increased presynaptic release probability at its glutamatergic synapses (Köhling et al., 2004). Additionally, autoradiographic studies showed decreased AMPA receptor binding in the dorsal parts of the striatum, which was interpreted as a counteraction to glutamatergic overactivity, and in the ventromedial thalamus. NMDA receptor binding was altered in different parts of the thalamus. However, most alterations were only found during dystonic attacks, suggesting that changed levels of glutamate may contribute to the occurrence of dystonia, but are not the primary defect of this disease (Nobrega et al., 2002, 1997).

In order to clarify if the antidystonic effect of the systemic administered glutamate receptor antagonists is based on an inhibition of an increased corticostriatal activity, we performed intrastriatal microinjections of different compounds which reduce the activation of glutamate receptors. The drugs were selected in accordance with previous studies in the dt^{sz} hamster (see above). We used D(−)-2-amino-5-phosphopentanoic acid (AP-5) as a competitive antagonist of the glutamate binding site of the NMDA receptors and 2,3-dioxo-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) for competitive antagonism of the AMPA receptors. (*R*)-(+)-3-amino-1-hydroxypyrrolidin-2-one (HA-966) was applied as a partial agonist of the glycine binding site with a low intrinsic activity at the NMDA receptor and dizocilpine (MK-801) as a non-competitive antagonist of the NMDA receptor.

2. Methods

2.1. Animals

The present experiments were carried out on dt^{sz} mutant hamsters which were obtained by a selective breeding as previously described in detail (Löscher et al., 1989; Richter and Löscher, 1998). All experiments were done in compliance with the German Animal Welfare Act.

2.2. Induction of dystonic attacks and severity-score of dystonia

The dt^{sz} hamsters develop reproducible dystonic attacks in response to stressful stimuli. In our experiments, the stressful stimuli were standardized by the so-called "triple stimulation technique", consisting of (1) catching the animal out of its home cage and placing it on a balance; (2) injection of the vehicle or drug; and (3) placing the animal in a new empty plastic cage. After this procedure, dt^{sz} hamsters develop a sequence of abnormal movements and postures which can be rated by the following severity-score-system (Löscher et al., 1989): *stage 1*, flat body postures; *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*, immobilization in a twisted, hunched posture with hind- and forelimbs tonically extended forward. After reaching the individual maximum stage the hamsters completely recover within 2 to 5 h.

2.3. Drugs

NBQX and (+)-HA-966 were purchased from Tocris (Bristol, UK), D(−)-AP-5 and (+)-MK-801 hydrogen maleate from Sigma-Aldrich (Saint Louis, USA). All drugs were freshly dissolved in isotonic saline prior to the experiments. The injection volume of bilateral microinjections into the dorsal striatum was 0.5 µl per hemisphere (see below). The doses of these drugs were chosen on the basis of several previous experiments on rodents (Kaur et al., 1997; Maldonado-Irizarry and Kelley, 1994; Mintz et al., 1999; Morrow et al., 1999). The doses were increased until unequivocal effects could be observed in dystonic hamsters.

2.4. Surgery and microinjections

For bilateral microinjections into the dorsal striatum, permanent stainless-steel guide cannulae (length: 12.2 mm,

inner diameter: 0.45 mm) were chronically implanted in groups of 6–10 mutant hamsters at an age of 28–32 days, as previously described (Hamann and Richter, 2002; Rehders et al., 2000). The anaesthetized hamsters (pentobarbital 70 mg/kg) were placed into a stereotaxic frame and guide cannulae were implanted bilaterally into the striatum according to the following coordinates (relative to bregma in mm): AP +1.5, L ±2.3, V −2.7. The guide cannulae were held in place with anchor screws and dental acrylic cement on the skull surface. Two to 3 days after surgery, microinjections in unanaesthetized hamsters were performed using an injection cannula (length 13.2 mm, inner diameter 0.2 mm) which was inserted through the guide cannula into the left and right striatum (V: −3.7 to bregma). The drug-solutions or the vehicle (for pre- and post-drug recordings) were bilaterally delivered in a volume of 0.5 µl per hemisphere at a rate of 0.1 µl per min by using a 0.5 µl microsyringe (Hamilton, USA). The application was verified by a transparent tube system which included an air-bubble surrounded coloured fluid-level and allowed us to observe the changing fluid-levels of the drug-solutions or the vehicle. The injection cannula was removed 5 min after the administration. Already during these first 10 min of the injection procedure the severity of dystonia and behavioural effects were noted.

2.5. Pharmacological examinations

The effects of the glutamate receptor antagonists on the severity of dystonia were examined in groups of 6–10 dystonic hamsters at an age of 33–42 days. As described above, dystonic attacks were induced by the procedure of triple stimulation. The glutamate receptor antagonists were injected into the dorsal striatum per microinjections (bilateral 0.5 µl per hemisphere) instead of the vehicle. For pre- and post-drug control recordings the animals received the same volume of the vehicle intrastriatally. The hamsters were observed for 3 h after the triple stimulation, because the individual maximum stage of dystonia (score rating system see above) is usually reached within this period. During this time the severity of dystonia, the latencies to the different stages and the side effects were observed. Locomotor activity was determined by a three-point score system, as used in previous examinations (Richter and Löscher, 1995).

The rater of the severity of dystonia was not informed about the treatment condition of the animals. Pre- and post-drug control trials were undertaken 2–3 days before and 2–3 days afer drug testing. Hamsters which reached severity scores which differed in more than two scales between pre- and post-drug trails were not considered for final evaluations. All control and drug trials were done at the same time of the day between 9:00 and 12:00 a.m.

2.6. Histology

After the pharmacological experiments, the hamsters were deeply anaesthetized with pentobarbital (100 mg/kg i.p.) and transcardially perfused. Coronal sections (40 µm) were Nissl-stained and the positions of the tip of the guide cannulae were determined according to the stereotaxic atlas of the hamster brain (Morin and Wood, 2001). Only animals with correctly placed guide cannulae in the dorsal striatum were considered for final evaluations of striatal drug effects.

2.7. Statistics

The Friedman test was used to calculate whether there were significant differences within the first, second and third hour between control and pre- and post-drug trails, respectively. The Wilcoxon signed rank test was used post hoc to determine the pairs which differed (pre-drug to drug/post-drug to drug). Only in case of significant changes between pre-drug and drug treatment as well as post-drug and drug treatment, an effect was considered to be significant (at least $P<0.05$). The significance of differences between drug efficacies of glutamate receptor antagonists injected alone in comparison to co-administration was evaluated by the Kruskal–Wallis test (at least $P<0.05$), the Mann–Whitney rank sum test was used post hoc. The observed changes in locomotor activity were descriptive and not statistically analysed.

3. Results

All microinjections considered for final evaluations were located in the dorsal part of the caudate-putamen (number of animals see Table 1).

Table 1
Effects on latency to onset of dystonia in dt^{sz} mutant hamsters

Dose (per hemisphere) [µg]	"Latency on" [min]			(*n*)
	Pre-drug	Drug	Post-drug	
NBQX				
0.03	3.3±0.7	3.9±0.9	3.7±0.8	7
0.08	8.3±2.0	14.8±3.3[b]	8.4±2.2	9
0.16	12.5±1.8	17.0±3.8	15.1±2.6	8
0.25	2.8±0.4	7.7±1.7[a]	3.3±0.6	6
AP-5				
0.50	4.3±0.6	7.8±1.3	6.1±2.0	10
1.00	10.8±1.8	11.7±1.5	11.9±3.3	10
(+)-HA-966				
10.00	4.9±0.9	10.9±4.2	3.3±0.7	7
20.00	6.4±2.2	6.0±0.7	4.4±0.5	8
MK-801				
4.00	5.4±1.0	8.6±4.6[a]	4.2±0.6	8
NBQX+AP-5				
0.08+0.50	3.8±0.8	8.7±3.0	3.3±0.4	6
0.16+1.00	5.3±1.1	4.8±0.9	3.8±0.8	9

Significant drug effects on latency to onset of dystonia after bilateral striatal injections in comparison with vehicle controls 2–3 days before (pre-drug) and after (post-drug) treatment with the glutamate receptor antagonists in dt^{sz} mutant hamsters. Latency was determined as the time to the first unequivocal signs of the dystonic attacks (stage 2). Data are shown as means±S.E. of the number of animals indicated (*n*). Significant differences to pre-drug *and* post-drug controls are marked by [a]($P<0.05$), [b]($P<0.01$).

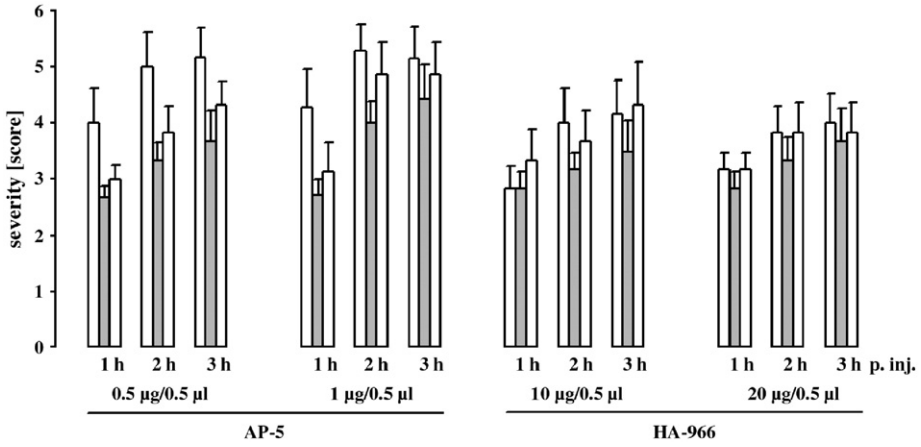

Fig. 1. Effects of the competitive NMDA receptor antagonist AP-5 (0.5 µg and 1.0 µg per hemisphere) and the partial agonist of the glycine binding site of the NMDA receptor (+)-HA-966 (10 µg and 20 µg per hemisphere) on the severity of dystonia in mutant hamsters after bilateral striatal microinjections. Usually, the individual maximum severity of dystonia is reached within 3 h after induction of dystonia by triple stimulation including the injection of drugs (grey bars) or vehicle for pre- and post-drug controls (white bars). The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd hour after administration of drug or vehicle, reflecting the progression of dystonia in dt^{sz} hamsters after treatment with the active compound and during control recordings. Control recordings were undertaken 2–3 days before (pre-drug control) and after (post-drug control) the drug trial. Data are shown as means + S.E. of 10 (AP-5, each dose), 7 ((+)-HA-966, 10 µg) or 8 ((+)-HA-966, 20 µg) dystonic hamsters.

The competitive NMDA receptor antagonist AP-5 (0.5 µg and 1 µg) and the partial agonist of the glycine binding site of the NMDA receptor (+)-HA-966 (10 µg and 20 µg) failed to exert significant effects on the individual maximum severity and on the progression of dystonia in the dt^{sz} hamster (Fig. 1). The latency to onset of dystonic movements remained unchanged (Table 1). Whereas the intrastriatal applications of 10 µg (+)-HA-966 and 0.5 µg AP-5 caused a slight hyperlocomotion during the first hour of observation, the injections of 20 µg (+)-HA-966 and 1 µg AP-5 induced an unequivocal hyperlocomotion (not illustrated) which lasted up to 3 h and was accompanied by ataxia and an increased grooming behaviour. Additionally, we observed a hypolocomotion within the first 30 min after injection of 1 µg AP-5 and marked facial contortions. The non-competitive NMDA receptor antagonist MK-801 caused no distinct effect on the severity of dystonic attacks at the injected dosage of 4 µg per hemisphere (not illustrated), but the latency to onset of dystonic movements was significantly prolonged in the hamster mutant ($P<0.05$, see Table 1). In respect of the severe side effects, such as hyperlocomotion, a moderate ataxia and an increased sensitivity to tactile impulses, we did not apply a higher dosage of MK-801.

The competitive AMPA receptor antagonist NBQX was injected at dosages of 0.03 µg, 0.08 µg, 0.16 µg and 0.25 µg per hemisphere (Fig. 2). The application of 0.03 µg caused no significant effect on the severity of dystonia as well as on the latency to onset of dystonic movements, whereas the injection of 0.08 µg NBQX per hemisphere reduced the individual maximum severity of dystonia in the dt^{sz} hamster in the third hour of observation ($P<0.01$) and prolonged the latency to onset of dystonic movements for about 5 min ($P<0.01$, see Table 1). Interestingly, the higher dosages of NBQX (0.16 µg and 0.25 µg) had no significant effect on the severity of dystonic attacks, only the latency to onset was retarded at the dosage of 0.25 µg per hemisphere ($P<0.05$). Whereas the application of 0.03 µg NBQX per hemisphere produced a slight hyperlocomotion in most animals, the injection of 0.08 µg first

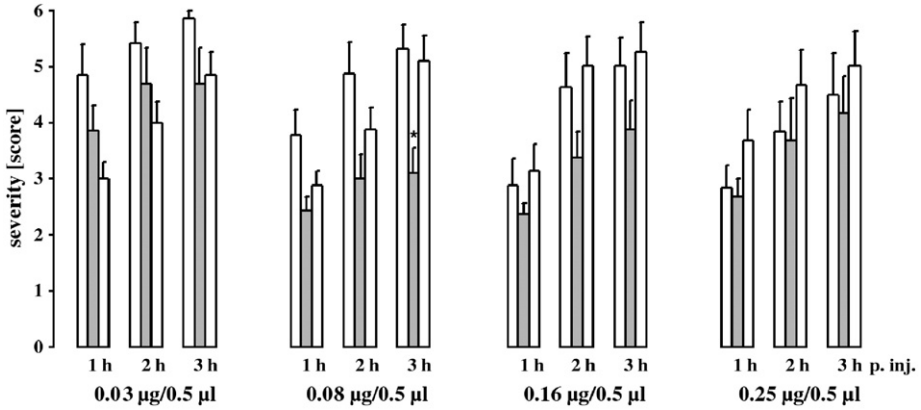

Fig. 2. Effect of the AMPA receptor antagonist NBQX on severity of dystonia in dt^{sz} hamsters after bilateral striatal microinjections. The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd hour after drug administration. Control recordings (white bars) were taken 2–3 days before (pre-drug control) and after (post-drug control) the drug trial (black bars). Data are shown as means + S.E. of 7 (0.03 µg), 9 (0.08 µg), 8 (0.16) or 6 (0.25) mutant hamsters. Significant differences to pre-drug *and* post-drug controls are marked by asterisks (*$P<0.01$). For further explanation see Fig. 1 legend.

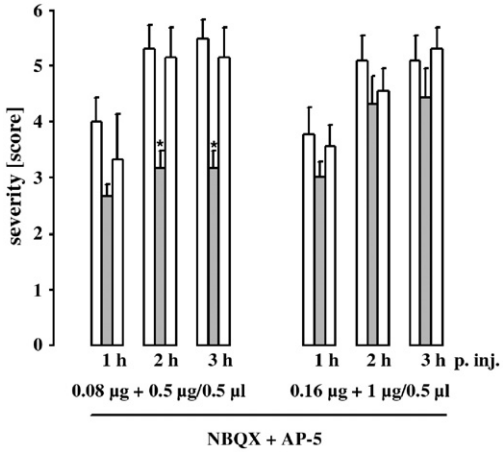

Fig. 3. Effect of combined bilateral striatal injections of AMPA receptor antagonist NBQX and the NMDA receptor antagonist AP-5 on severity of dystonia in dt^{sz} hamsters. The figure shows the average of the maximum individual severity scores of dystonia reached within the 1st, 2nd and 3rd hour after drug administration. Control recordings (white bars) were taken 2–3 days before (pre-drug control) and after (post-drug control) the drug trial (black bars). Data are shown as means+S.E. of 6 (0.08 µg+0.5 µg) or 9 (0.16 µg+1.0 µg) mutant hamsters. Significant differences to pre-drug *and* post-drug controls are marked by asterisks (*$P<0.05$). For further explanation see Fig. 1 legend.

caused a slight hypolocomotion during the first hour of observation, which was replaced by a moderate hyperlocomotion during the second hour and accompanied by ataxia. The hypolocomotion was not observed after the application of higher dosages, but most animals exhibited a moderate hyperlocomotion and ataxia.

The combined application of the NMDA receptor antagonist AP-5 (0.5 µg) and the AMPA receptor antagonist NBQX (0.08 µg) significantly reduced the severity of dystonia in the hamster mutant during the second and the third hour of observation ($P<0.05$, see Fig. 3). In comparison to the single administration, there was no synergistic effect of the combination. The latency to onset of dystonic movements remained unchanged. Along with the single application of 0.16 µg NBQX, the combination with 1 µg AP-5 did not exert a significant effect on the severity of dystonia as well as on the latency to onset of dystonia. Expect for the lack of hypolocomotion, the combined application of the drugs caused similar behavioural effects to the single injections.

4. Discussion

The striatum plays a critical role in regulating the activity flow in circuits involving the cortex, the basal ganglia, and the thalamus. Its major input derives from glutamatergic afferents of the cortex. Previous studies suggested pathophysiological relevant alterations of this corticostriatal pathway in the dt^{sz} hamster mutant. Therefore, we investigated the effect of different antagonists of ionotropic glutamate receptors after striatal microinjection on the severity of dystonia in this animal model.

As in previous studies of systemic administrations of the AMPA receptor antagonist NBQX (Richter et al., 1993), its striatal injection reduced the severity of dystonic attacks in the dt^{sz} hamster. In contrast to the dose-dependent beneficial effects after systemic application, in the present study the antidystonic effect disappeared after intrastriatal injections of higher dosages. This discrepancy of the two application forms suggests that the beneficial effect of the systemically administered NBQX was at least partly mediated by extrastriatal brain regions. Previous autoradiographic studies demonstrated a reduced AMPA receptor binding in dt^{sz} hamsters during dystonic attacks in several striatal subregions, but also in other brain regions, e.g. limbic structures and the cerebral cortex, which was interpreted as a down regulation in response to an glutamatergic overactivity (Nobrega et al., 2002). As the activity of the entopeduncular nucleus was found to be reduced in the hamster mutant, probably leading to a disinhibition of the thalamus (Gernert et al., 2000), the antidystonic effect of the AMPA receptor antagonist after systemic treatment could have been mediated by an inhibition of an overactive thalamocortical transmission. The thalamic nuclei which are innervated by the entopeduncular nucleus are the main route by which the basal ganglia influence motor areas of the cerebral cortex (Crossman, 2000). An increased thalamic activity is proposed to facilitate motor pattern generators in the cerebral cortex (Mink, 2003) and could therefore contribute to the occurrence of dystonic attacks.

Nevertheless, the striatal application of NBQX reduced the severity of dystonia at one dosage and this effect proved to be reproducible in combination with AP-5, even though the NMDA receptor antagonist was not able to increase the effect of NBQX significantly. This antidystonic effect suggests that corticostriatal overactivity could possibly contribute to the manifestation of dystonic attacks in the hamster mutant, but the lack of dose-dependence weakens this assumption. The dosage of 0.03 µg NBQX per hemisphere was probably too low to affect the motor disturbance, which is indicated by the absence of behavioural effects after its application. The lack of antidystonic effects of the higher dosages 0.18 and 0.25 µg of NBQX, which induced behavioural effects, might be related to effects on other types of striatal neurons.

The striatum mainly consists of GABAergic projection neurons, which constitute about 95% of the striatal neurons and moderately express different AMPA receptor subunits (Chan et al., 2003). The remaining neurons are cholinergic and GABAergic interneurons, including parvalbumin-, calretinin- and nitric oxide synthase-reactive interneurons (Kawaguchi, 1997) which were found to be reduced in the dt^{sz} hamster (Gernert et al., 2000; Hamann et al., 2005; Sander et al., 2006). Inhibition of these GABAergic interneurons could be a reason for the observed lack of an antidystonic effect of NBQX at the doses of 0.16 µg and 0.25 µg. However, the nitric oxide synthase-reactive interneurons barely express AMPA receptor subunits and there is few known about the physiological role calretinin-reactive interneurons within the striatum. By contrast, the parvalbumin-reactive interneurons highly express all types of AMPA receptor subunits (Bernard and Bolam, 1998; Chan et al., 2003). Although providing only 3–5% of the striatal cell population (Kawaguchi, 1997), they represent the major inhibitory source within the striatum and counteract the

glutamatergic corticostriatal activation of GABAergic projection neurons. In view of their comparatively low fraction, the higher dosages of NBQX (0.18 and 0.25 μg per hemisphere) possibly completely antagonised the AMPA receptors on striatal parvalbumin-reactive interneurons in the *dt*^{sz} hamster, resulting in an intense interference of the feed-forward inhibition. This could be relevant for the disappearance of the beneficial effects of NBQX at these doses.

All striatal injected NMDA receptor antagonists failed to affect the severity of dystonic attacks in the *dt*^{sz} hamster, although significant side effects of the drugs indicated adequate chosen dosages. These results are in line with previous autoradiographic studies, which did not show alterations of NMDA-receptor binding within the striatum of *dt*^{sz} hamsters (Nobrega et al., 1997). Therefore, the antidystonic effects of the systemic administered NMDA receptor antagonists (Löscher and Richter, 1993; Richter et al., 1991) seem to be mediated by extrastriatal brain regions, too.

On the other hand, previous electrophysiological recordings of brain slices have demonstrated an increased excitability of the corticostriatal pathway in *dt*^{sz} hamsters (Köhling et al., 2004). As discussed above, the glutamatergic influence on striatal AMPA receptors may be somewhat underestimated by the results of our studies. The lack of an antidystonic effect of the administered NMDA receptor antagonists could be explained by the predominant role of AMPA receptors in the generation of striatal excitatory synaptic potentials. There is evidence, that a major component of the excitatory synaptic potential recorded from striatal neurons is mediated by AMPA receptors (Calabresi et al., 1996) and several studies showed only a weak reduction or even a failure to inhibit cortically evoked excitatory synaptic potentials by NMDA receptor antagonists (Herrling, 1985; Vilagi et al., 1998).

In view of previous studies, which demonstrated a clear antidystonic effect of the combined intrastriatal microinjection of dopamine D_1- and D_2-receptor antagonists (Rehders et al., 2000), a nigrostriatal dopaminergic overactivity is possibly more important for the manifestation of dystonic attacks in the *dt*^{sz} hamster. Its pathophysiological relevance is supported by results of recent microdialysis studies, which showed increased striatal levels of extracellular dopamine and its metabolites in hamsters during dystonic episodes (Hamann and Richter, 2004). Measurements of striatal extracellular amino acid levels, including aspartate and glutamate, are under the way to examine if temporal increases of excitatory amino acids could be involved in the manifestation of dystonia, as suggested by electrophysiological studies and the NBQX effects (see above).

In summary, the striatal manipulations of ionotropic glutamate receptors were less effective than assumed by previous electrophysiological studies and neurochemical examinations of the striatal glutamatergic system in *dt*^{sz} hamsters. However, the AMPA receptor antagonist NBQX exerted reproducible antidystonic effects at one dosage, indicating a pathophysiological relevant implication of this receptor type in the occurrence of dystonic attacks in the hamster mutant. The beneficial effects of the systemic administered AMPA and NMDA receptor antagonists of foregoing studies are probably mediated, at least partly, by extrastriatal brain regions. Nevertheless, glutamate receptors still represent a seminal target for new approaches in the medical treatment of dystonia. New insights into the role of the different striatal glutamate receptor subtypes in processing corticostriatal input are necessary for a further understanding of the pathophysiological mechanisms of the manifestation of dystonic episodes.

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