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European Journal of Pharmacology 443 (2002) 59–70

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Effects of striatal injections of $GABA_A$ receptor agonists and antagonists in a genetic animal model of paroxysmal dystonia

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Received 20 December 2001; received in revised form 11 March 2002; accepted 19 March 2002

Abstract

The underlying mechanisms of idiopathic dystonias are poorly understood. The dystonic phenotype in the dt^{sz} mutant hamster, a model of paroxysmal dystonia, has been suggested to be based on a deficit of γ-aminobutyric acid (GABA)ergic interneurons and changes of the $GABA_A$-benzodiazepine receptor complex in the striatum. In order to confirm and extend previous observations, the effects of compounds which bind to different sites of the $GABA_A$ receptor on the severity of dystonia were determined after striatal microinjections in comparison to systemic treatments in dt^{sz} mutants. The $GABA_A$ receptor agonist (muscimol) and the benzodiazepine (flurazepam) reduced the severity of dystonia after striatal and systemic injections. The antidystonic effects of the barbiturate phenobarbital were less marked both after striatal and intraperitoneal administration of drugs. Intrastriatal injections of GABA delayed the onset of dystonic attacks. Striatal and systemic treatments with the $GABA_A$ receptor antagonist, bicuculline, and with pentylenetetrazole, which reduces GABAergic function, accelerated the onset of dystonia at subconvulsant doses. The benzodiazepine receptor antagonists flumazenil aggravated dystonia after systemic and intrastriatal injections. In all, the present data substantiate the relevance of striatal GABAergic disinhibition in the pathogenesis of paroxysmal dystonia in dt^{sz} mutants. © 2002 Published by Elsevier Science B.V.

Keywords: Basal ganglion; Dyskinesia; Movement disorder; Striatum

1. Introduction

Dystonia, characterized by involuntary and sustained contractions of opposing muscles, frequently causing patterns of twisting movements or abnormal postures, is regarded as a basal ganglia disorder (Fahn et al., 1998; Vitek and Giroux, 2000). Symptomatic dystonias are often associated with lesions in the basal ganglia, particularly in the striatum, while primary dystonias occur in the absence of lesions as determined by standard techniques (Bhatia and Marsden, 1994). Dependent on the phenotypic and genotypic subtypes, idiopathic dystonias are likely related to different brain abnormalities which is reflected by the distinct response to drugs (Fahn, 1995). Throughout the few clearly defined animal models, the dt^{sz} mutant hamster shows the phenomenological characteristics of human primary paroxysmal non-kinesiogenic dystonic choreoathetosis (brief: paroxysmal dystonia), in which long-lasting attacks of dyskinesia can be provoked by stress and caffeine (Demirkiran and Jankovic, 1995; Richter and Löscher, 1998). In view of the precipitating factors of dystonic episodes in humans, i.e., stress and caffeine which enhance the dopaminergic activity, paroxysmal dystonia is possibly related to dopaminergic dysfunctions (Todd and Perlmutter, 1998). Neuroleptics can, in fact, improve paroxysmal dystonia in humans and in dt^{sz} hamsters (Przuntek and Monninger, 1983; Richter and Löscher, 1993). Although the dopaminergic system is anatomically intact in dystonic hamsters (Nobrega et al., 1999), altered dopamine receptor binding and striatal microinjections of dopamine receptors agonists and antagonists indicated that striatal dopaminergic overactivity plays a critical role for the manifestation of dystonia (Nobrega et al., 1996; Rehders et al., 2000).

In contrast to the hypothesis that dysfunction of the dopaminergic system is the culprit in dystonias (Todd and Perlmutter, 1998), recent findings in dt^{sz} mutant hamster suggested that striatal dopaminergic overactivity is merely the result of γ-aminobutyric acid (GABA)ergic disinhibition. While histological standard techniques failed to disclose any

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0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0014-2999(02)01546-7

pathomorphological alterations within the central nervous system in dt^{sz} mutant hamsters (Wahnschaffe et al., 1990), a significant deficit of striatal parvalbumin-immunoreactive GABAergic interneurons was found by immunohistochemical methods (Gernert et al., 2000). In accordance with this structural defect, decreased GABA levels and a reduced expression of the GABA synthesizing enzyme were detected in the striatum of these animals (Burgunder et al., 1999; Löscher and Hörstermann, 1992). Probably based on a GABAergic disinhibition, the neuronal activity is increased within the striatum of dt^{sz} mutants (Gernert et al., 1999; Richter et al., 1998), leading to a reduced basal ganglia output (Bennay et al., 2001; Gernert et al., 2000).

Systemic administrations of GABA-potentiating drugs, such as the benzodiazepine diazepam, exerted beneficial effects in mutant hamsters (Fredow and Löscher, 1991). Benzodiazepines are also the most effective drugs in patients with paroxysmal dystonia (Demirkiran and Jankovic, 1995). With regard to the systemic effects of diazepam in mutant hamsters, the finding of an enhanced affinity and density of benzodiazepine binding sites in the striatum of dt^{sz} mutants (Pratt et al., 1995) has been interpreted as an upregulation of these sites. However, this has not so far been examined by striatal pharmacological manipulations of the $GABA_A$–benzodiazepine receptor complex. Previous pharmacological examinations of the GABAergic system in mutant hamsters were restricted to systemic treatments (Richter and Löscher, 1998) except intrastriatal injections of two doses of muscimol (Gernert et al., 2000). The data of the first experiments with muscimol are included in the present study to allow comparisons with further doses of muscimol and with the effects of other compounds.

Although most changes in the GABAergic system were detected within the striatum of dystonic hamster, increased binding at the picrotoxinin site of the $GABA_A$ receptor complex was found in "extrastriatal" regions, e.g., cortical areas and thalamic nuclei (Nobrega et al., 1995; Richter and Löscher, 1998). The aim of the present study was to examine if striatal manipulations of different binding sites of the $GABA_A$ receptor complex confirm the importance of neurochemical and neuroanatomical changes of the GABAergic system within the striatum for the occurrence of paroxysmal dystonia in the dt^{sz} mutant hamster. The efficacy of drugs which potentiate or inhibit $GABA_A$ receptor-mediated inhibition was therefore determined after striatal microinjections in comparison with systemic administrations. The compounds were injected into the dorsal part of striatum, as done in recent examinations of the dopaminergic system in mutant hamsters (Rehders et al., 2000), to allow comparison between the effects of manipulations of both systems. In this study, we examined the response to drugs which interact with distinct binding sites of the $GABA_A$ receptor complex, i.e., GABA, benzodiazepine, barbiturate and picrotoxinin binding sites (Möhler, 1992; Sieghart, 1992).

2. Methods

2.1. Animals

Experiments were carried out in male and female dt^{sz} mutant Syrian golden hamsters, which were obtained by selective breeding as previously described in detail (Fredow and Löscher, 1991; Löscher et al., 1989). In mutant hamsters, motor disturbances are transmitted by a recessive gene (Richter and Löscher, 1998). In cases of unexpected behavioral effects of drugs in dt^{sz} mutants, non-dystonic control hamsters were used. These animals were obtained by breeding pairs which were provided by a commercial breeder (Central Institute for Laboratory Animal Breeding, Hannover, Germany). All hamsters were born and kept under the same controlled environmental conditions. The experiments were done in compliance with the German Animal Welfare Act.

As previously described in detail (Richter and Löscher, 1998), motor impairments in dt^{sz} hamsters show the clinical and pharmacological characteristics of human primary paroxysmal non-kinesiogenic dystonic choreoathetosis in which long-lasting dystonic attacks can be induced by stress. Dystonia in dt^{sz} mutants shows an *age-dependent time course* with first occurrence at age of about 16 days. Dystonic symptoms (severity score see below) reach a maximum between 30 and 42 days (*max-period*: suitable to detect beneficial drug effects). Thereafter, the severity of dystonia slowly declines (50 to about 60 days of life; *postmax-period:* suitable to observe drug-induced aggravation of dystonia). Complete remission of stress-inducible dystonic attacks occurs at an age of about 10 weeks (Löscher et al., 1995).

2.2. Induction of dystonic attacks and severity score of dystonia

In mutant hamsters, dystonic attacks can be reproducibly induced by a triple stimulation technique (Löscher et al., 1989; 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) injection of saline (or vehicle or drug, see Section 2.5), and (3) placement of the animal in a new plastic cage. After this procedure, dt^{sz} hamsters develop a pattern of abnormal movements and postures. Since the symptoms occur in a constant sequence, the severity of dystonia can be rated by following score system (Löscher et al., 1989): 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. The individual maximum stage of dystonia is usually reached within 3 h after the hamsters

were placed in the new cage. After reaching the individual maximum stage, the hamsters recover within 2–5 h.

In the present study, all animals were repeatedly tested by triple stimulations (injections of isotonic saline) every 2–3 days after weaning (21 days of life) until the severity of dystonia was determined to be reproducible. Thereafter, the effects of drug on the severity of dystonia were examined during the max-period (33–42 days) and/or during the postmax-period (50–65 days of life). As observed in several previous studies (e.g., Rehders et al., 2000), latency to onset of dystonic attacks usually increases in aged animals, but is usually reproducible during max and postmax life-periods. In order to determine if GABA potentiation can delay the onset of dystonia, animals with a short latency to onset of dystonia during pre-drug testing were preferred for treatments with compounds which enhance GABAergic inhibition.

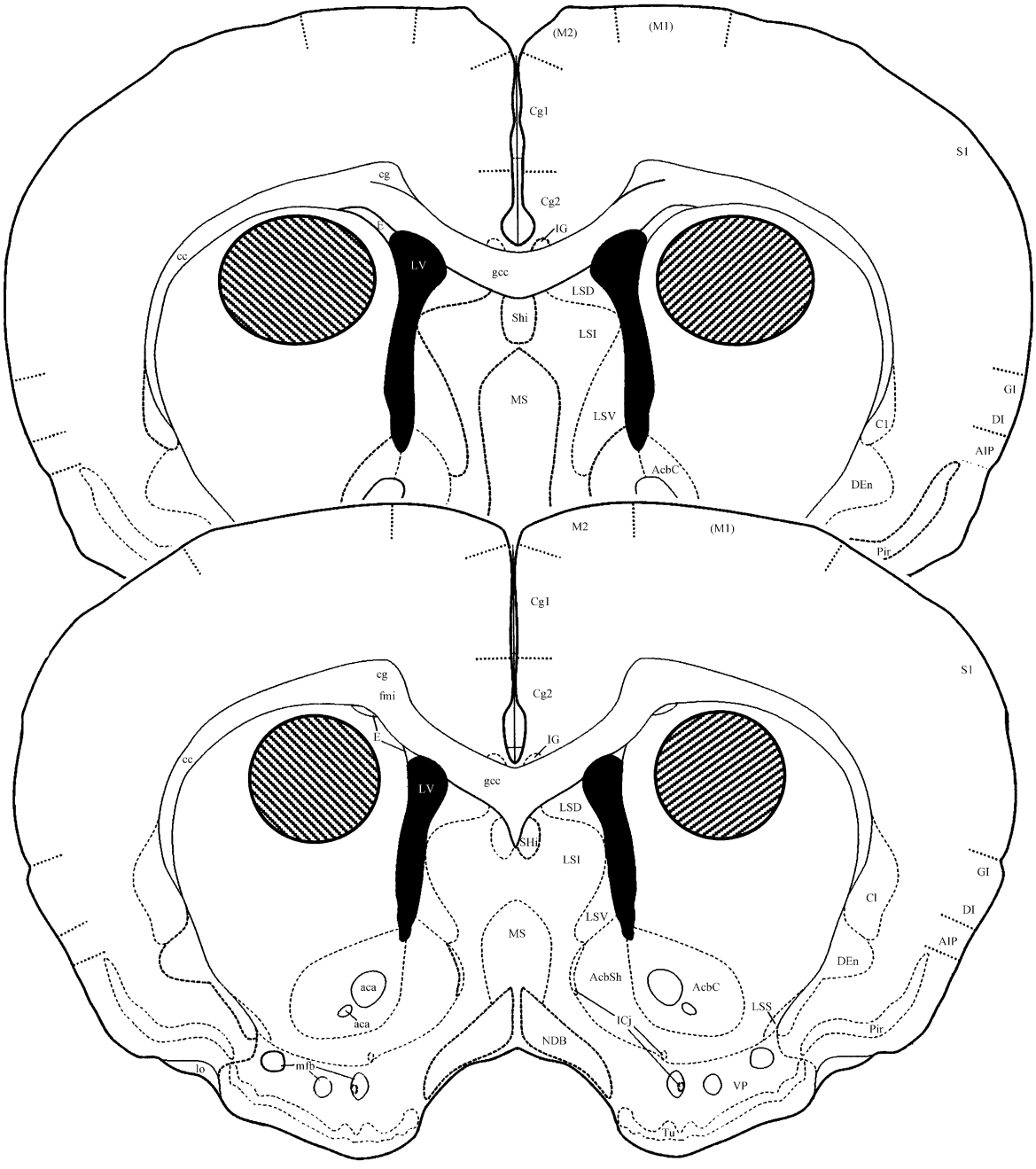

Fig. 1. Localization of the guide cannulae for microinjections of compounds which enhance or inhibit $GABA_A$ receptor-mediated inhibition in mutant hamsters (total number: 128). As histologically verified, all tips of chronically implanted guide cannulae were located within the stripped area in the dorsal striatum (1.5 and 1.8 mm to bregma). This type of localization is comparable to recent pharmacological manipulations of the striatal dopaminergic system in mutant hamsters (Rehders et al., 2000).

2.3. Drugs

In view of intrastriatal microinjections, compounds with sufficient water solubility were preferred. The $GABA_A$ receptor agonist muscimol was purchased from Tocris (Bristol, UK). GABA, the $GABA_A$ receptor antagonist bicuculline, the benzodiazepine flurazepam, the barbiturate phenobarbital and pentylenetetrazole, which disturbs GABAergic inhibition by binding at the picrotoxinin site (Möhler, 1992; Olsen, 1982; Sieghart, 1992), were purchased from Sigma (Steinheim, Germany). These compounds were freshly dissolved in isotonic saline. Flumazenil, provided by LaRoche (Basel, Switzerland), was dissolved in 5% (intrastriatal injections) or 20% tetraglycol (systemic administrations) prior the experiments. For systemic treatments, all drugs or vehicles (for control recordings) were administered intraperitoneally (i.p.) at an injection volume of 5 ml/kg. The injection volume of bilateral microinjections into the dorsal striatum (intrastriatal) was 0.5 μl per hemisphere (see below).

The doses of these drugs for systemic and striatal treatments were chosen based on several previous experiments in rats (e.g., De Beltran et al., 1993; Turski et al., 1990). Doses were increased until unequivocal behavioral effects could be observed in dystonic hamsters. This criterion was fulfilled for all used compounds after the different routes of administration. Compounds which disturb GABAergic inhibition were given at subconvulsant doses. Systemic treatments with pentylenetetrazole were restricted to 25 mg/kg because the effects of higher doses after intraperitoneal injections were already tested in a previous study (Fredow and Löscher, 1991).

2.4. Surgery and microinjections

As recently described for striatal manipulations of the dopaminergic system in mutant hamsters (Rehders et al., 2000), permanent stainless-steel guide cannulae (length: 12.7 mm, inner diameter: 0.4 mm) were chronically implanted into the left and right dorsal striatum in groups of 5–12 mutant hamsters at age 30–32 days for bilateral microinjections. In each anaesthetized animal (pentobarbital 60 mg/kg), bilateral guide cannulae were implanted into the striatum according to the following coordinates (relative to bregma in mm): AP +1.5, L ±2.1, V −2.4, which were experimentally determined according to the method of Paxinos and Watson (1986) in preceding experiments (Rehders et al., 2000). The guide cannulae were held in place with anchor screws and dental acrylic cement on the skull surface. Two to three days after surgery, microinjections into the striatum of unanesthetized hamsters were performed using an injection cannula (length of 13.7 mm, inner diameter of 0.2 mm), which was inserted through the guide cannula into the left and right striatum (V: −2.7 mm to bregma). The drug

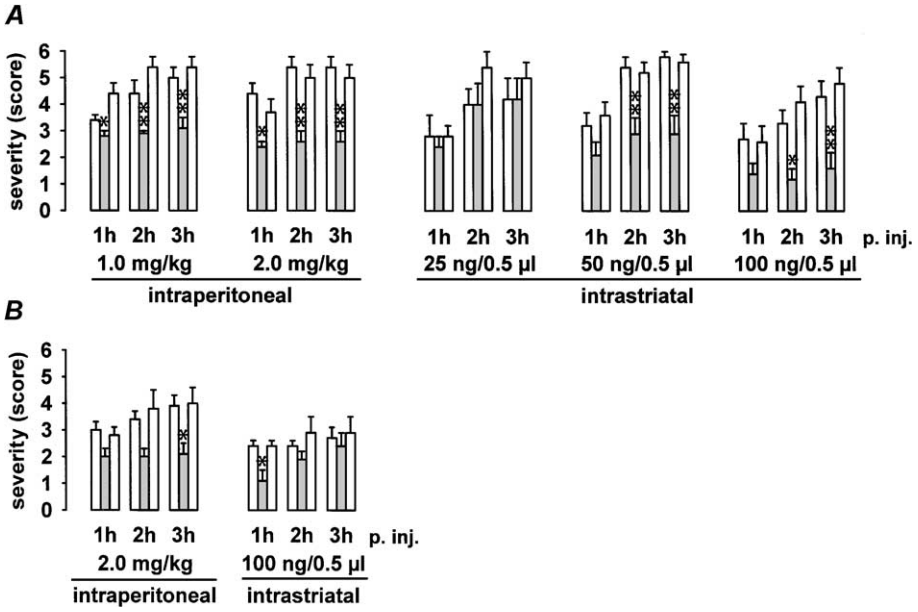

Fig. 2. Effects of $GABA_A$ receptor agonist muscimol on the severity of dystonia in mutant hamsters at an age of most marked expression of dystonia (maxperiod; A) and in older animals (postmax; B) after systemic administration (1.0 and 2.0 mg/kg, i.p.) and after bilateral striatal microinjections (25, 50 and 100 ng/0.5 μl/hemisphere). White bars in each set of three bars indicate the control values obtained 2 days before drug administration (first white bar) and 2 days after drug administration (second white bar). Gray bar refers to the day of drug administration. The individual maximum severity of dystonia is usually reached within 3 h after induction of dystonia by triple stimulation including injection of drugs or vehicle. Figure shows the average of the maximum individual severity scores of dystonia reached within 1, 2 and 3 h post-injection (p. inj.) of vehicle or muscimol. Asterisks indicate significant improvement of dystonia in comparison to pre- *and* post-drug control (* $P<0.05$, ** $P<0.01$). Data are shown as means + S.E. of 5 (25 ng), 7 (100 ng) or 9 (50 ng, 1 and 2 mg/kg) dystonic hamsters.

solutions or 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/min. The injection cannula was removed 5 min after the administration. Already within the first 10 min of the injection procedure, the behavioral effects and the effects on dystonia were noted.

2.5. Pharmacological treatments

Drug effects on the severity of dystonia were examined in groups of 5–12 dystonic hamsters at the age of maximum severity of dystonia (33–42 days) and/or at age 50–65 days. Each group was used for 1–3 drug trails. In cases of repeated testing, the drug-free interval was at least 4 days. Dystonic attacks were induced by the procedure of triple stimulation, as described above, but instead of saline, vehicle (control trials) or active compounds were injected i.p. (injection volume: 5 ml/kg) or into the dorsal striatum per micro-injections (bilateral 0.5 μl/hemisphere). For pre- and post-drug control recordings, the animals received the same volume of vehicle i.p. or into the striatum, respectively. Since the individual maximum stage of dystonia (score rating system, see above) 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. Locomotor activity was determined by a score system, as used in previous examinations (e.g., Richter and Löscher, 1995). Other adverse effects were not quantified. The rater of the severity of dystonia was blind to the treatment condition of the animals. Pre- and post-drug control trials were undertaken 2 days before and 2 days after drug testing. 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 examination of striatal drug effects, the hamsters were deeply anaesthetized with pentobarbital (100 mg/kg i.p.) and transcardially perfused with phosphate-buffered saline followed by 4% phosphate-buffered formaldehyde. Coronal sections of the brains (40 μm) were Nissl-stained and the positions of the tip of the guide cannulae were determined according to the stereotaxic atlas of the golden hamster brain (Morin and Wood, 2001). As shown in Fig. 1, only animals with correct placement of the guide cannulae in the dorsal striatum were considered for final evaluations of striatal drug effects.

2.7. Statistics

The significance of differences in the severity of dystonia as well as in latencies to onset of dystonia between control trials (pre- and post-drug) and drug trial in the same group of animals was calculated by Friedman test and, 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.

3. Results

All microinjections considered for final evaluations were done in the dorsal part of the caudate–putamen, as shown in Fig. 1. The microinjections did not cause striatal lesions. Antidystonic drug effects are preferentially illustrated for the determinations during the life-period of most marked expression of dystonia (max-period, 33–42 days), while

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

Dose	Age (days)	Latency (min)			(*n*)
		Pre-drug	Drug	Post-drug	
GABA potentiation					
Muscimol					
1.0 (i.p.)	36–39	6.3 ± 0.9	8.3 ± 1.0[a]	5.4 ± 0.7	9
25 ng (i.st.)	33–42	6.6 ± 1.5	22.2 ± 3.6[a]	7.4 ± 1.4	5
50 ng (i.st.)	33–42	11.6 ± 1.8	33.3 ± 7.2[b]	7.1 ± 0.6	9
100 ng (i.st.)	50	5.7 ± 0.4	40.0 ± 15.9[b]	5.7 ± 0.5	7
Flurazepam					
100 ng (i.st.)	40	4.9 ± 0.6	8.1 ± 0.9[b]	7.4 ± 1.6	9
200 ng (i.st.)	38–42	5.1 ± 0.5	12.4 ± 3.2[b]	4.0 ± 0.3	9
	55	5.9 ± 0.5	13.6 ± 2.3[a]	8.0 ± 1.4	9
Phenobarbital					
100 ng (i.st.)	36	4.4 ± 0.3	12.5 ± 2.2[a]	8.6 ± 1.4	8
	50	7.1 ± 1.3	26.0 ± 7.3[a]	7.6 ± 0.6	9
150 ng (i.st.)	39	6.1 ± 0.5	25.6 ± 6.4[b]	5.4 ± 0.3	10
GABA					
100 μg (i.st.)	41	5.1 ± 0.6	18.6 ± 7.9[a]	5.0 ± 0.6	8
200 μg (i.st.)	55	6.9 ± 0.7	42.9 ± 7.2[b]	6.1 ± 0.5	8
GABA inhibition					
Bicuculline					
1.5 (i.p.)	35	12.0 ± 1.4	7.0 ± 2.0[b]	14.8 ± 1.8	9
50 ng (i.st.)	62	14.3 ± 3.2	5.0 ± 1.1[a]	10.0 ± 1.9	6
100 ng (i.st.)	59–65	16.8 ± 4.7	3.9 ± 0.2[c]	14.3 ± 3.1	12
150 ng (i.st.)	52–58	7.3 ± 0.9	2.7 ± 0.2[c]	11.9 ± 1.5	11
Flumazenil					
5.0 (i.p.)	36–38	13.8 ± 2.8	5.2 ± 0.8[c]	11.4 ± 0.7	12
	52–59	13.5 ± 2.1	7.5 ± 1.4[b]	17.0 ± 1.6	11
10.0 (i.p.)	41	11.1 ± 0.8	4.6 ± 0.5[c]	15.3 ± 1.0	12
	57	18.7 ± 1.7	15.3 ± 2.2[b]	26.6 ± 4.0	12
100 ng (i.st.)	56–62	22.4 ± 8.0	4.7 ± 0.6[b]	14.9 ± 1.4	10
Pentylenetetrazole					
25 (i.p.)	35	12.0 ± 1.0	1.7 ± 0.3[b]	6.9 ± 1.1	10
	50	26.0 ± 3.0	2.3 ± 0.2[a]	15.0 ± 3.7	7
0.7 μg (i.st.)	56–63	14.7 ± 3.8	4.6 ± 0.3[b]	19.1 ± 6.9	10
1.4 μg (i.st.)	37–38	5.3 ± 0.7	3.6 ± 0.3[a]	7.1 ± 1.9	9
	58–61	15.4 ± 7.2	4.9 ± 0.4[b]	8.9 ± 1.1	8

Significant effects of drug on latency to onset of dystonia after intraperitoneal (i.p., mg/kg) or bilateral striatal (i.st., ng or μg/hemisphere) injections in comparison to vehicle controls 2 days before (pre-drug) or 2 days after (post-drug) treatment with active compounds in dt^{sz} mutant hamsters at different ages. 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]($P<0.01$), [c]($P<0.001$).

figures showing prodystonic effects are restricted to findings in mutant hamsters which had passed the age of most severe dystonia (postmax-period, 50–65 days).

As shown in Fig. 2, the selective $GABA_A$ receptor agonist muscimol exerted striking antidystonic effects after systemic (i.p.) and intrastriatal injections in dt^{sz} mutants. The figure shows the average of maximum individual severity scores of dystonia reached within 1, 2 and 3 h after administration of muscimol or vehicle, reflecting the progression of dystonia in dt^{sz} hamsters after treatment with the active compound and during control recordings. The effects of muscimol (1, 2 mg/kg and 50, 100 ng) on the maximum severity of dystonia (third hour) during the max-period have been included in a recent study (Gernert et al., 2000). Apart from a reduction of the maximum severity of dystonia (third hour of observation) after treatment with 1 and 2 mg/kg, i.p., or 50 and 100 ng/hemisphere (Gernert et al., 2000), muscimol delayed the progression of dystonic attacks (Fig. 2A, Table 1). Already at a dose of 25 ng/hemisphere, muscimol significantly increased the latency to onset of dystonia (Table 1). Furthermore, the present data show that muscimol exerts antidystonic activity also in mutant hamsters which had passed the age of most marked expression of paroxysmal dystonia (Fig. 2B, Table 1). In mutant hamsters (max-period), adverse effects were hyperlocomotion after administration of 1 mg/kg, i.p. and striatal injections of 25 and 50 ng/hemisphere, while higher doses of 2 mg/kg i.p. or 100 ng/hemisphere caused moderate sedation and reduced locomotor activity. These adverse effects lasted from 10 to 180 min after injection of various doses and administration routes. The behavioral effects were similar in a group of six age-matched non-dystonic control hamsters. In older dt^{sz} mutants (postmax-period), muscimol did not affect the locomotor activity after systemic administrations of 2 mg/kg and only a short-lasting (40 min), moderate hypolocomotion could be observed after striatal injections of 100 ng.

The benzodiazepine flurazepam significantly decreased the severity of dystonia at doses of 2.5 and 5 mg/kg in mutant hamsters at the age of most marked expression of dystonia (Fig. 3) and in older animals (postmax, not illustrated). While systemic treatments with this benzodiazepine failed to exert significant effects on latency to onset, striatal injections of 100 ng (max-period) and 200 ng/hemisphere (max- and postmax) delayed the occurrence of first dystonic symptoms (Table 1). Intrastriatal injections of 50- and 100-ng doses did not exert significant effects on the severity of dystonia. However, significant beneficial effects could be observed after the administration of 200 ng flurazepam in mutant hamsters at age 38–42 days (max-period: Fig. 3), but not in older animals (postmax: not illustrated). In dystonic animals (max- and postmax-period) and in non-dystonic control hamsters, flurazepam provoked moderate to marked hyperlocomotion, which lasted from 30 to 130 (2.5 mg/kg) or 180 (5 mg/kg) min after i.p. administration. In contrast, depressant effects on motor activity and sedation, lasting from 5 to 180 min, were caused by striatal injections of 100- and 200-ng doses in mutant hamsters at age 38–42 days, while older animals exhibited hyperlocomotion after treatments with 200 ng. A dose 50 ng did not exert any behavioral effects.

The barbiturate phenobarbital exerted moderate antidystonic effects at doses of 20 and 30 mg/kg, i.p., during the max-period (Fig. 4). In contrast to systemic treatments, striatal injections of phenobarbital retarded the onset of

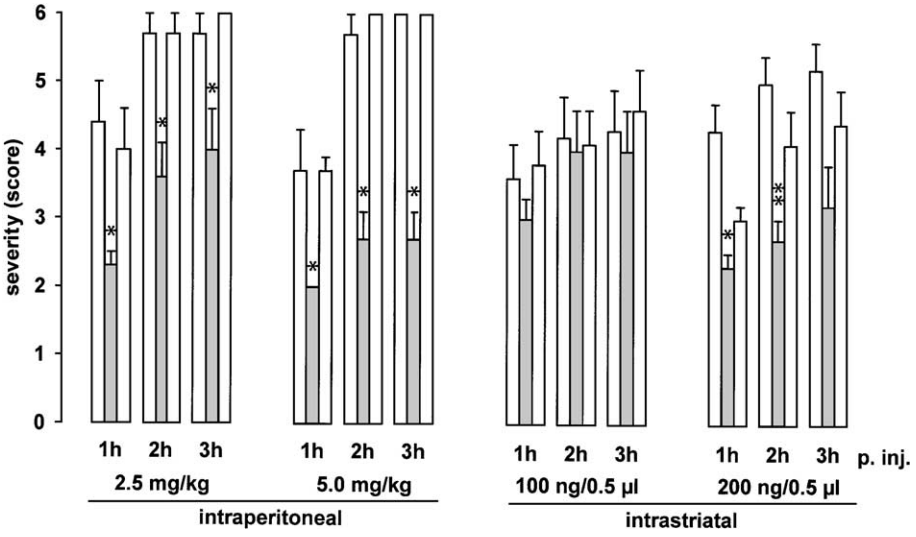

Fig. 3. Effect of the benzodiazepine flurazepam on the severity of dystonia in mutant hamsters after systemic administration (2.5 and 5.0 mg/kg, i.p.) and after bilateral striatal microinjections (100 and 200 ng/0.5 µl/hemisphere). The figure shows the average of maximum individual severity scores of dystonia reached within 1, 2 and 3 h after administration of flurazepam. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant improvement of dystonia in comparison to pre- *and* post-drug control ($*P<0.05$, $**P<0.01$). Data are shown as means + S.E. of 6 (2.5 mg/kg), 7 (5.0 mg/kg) or 9 (100 and 200 ng) dt^{sz} hamsters. For further explanation, see Fig. 2 legend.

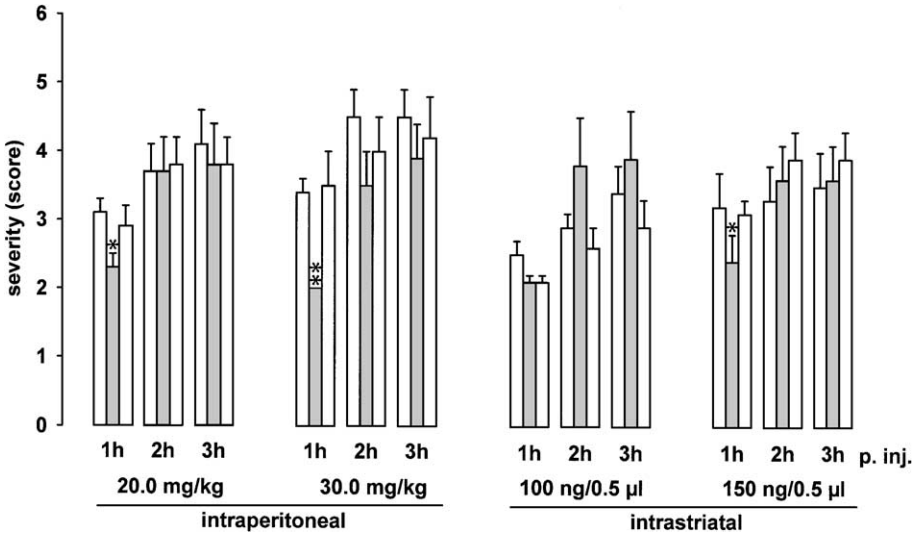

Fig. 4. Effect of the barbiturate phenobarbital on the severity of dystonia in mutant hamsters after systemic administration (20.0 and 30.0 mg/kg, i.p.) and after bilateral striatal microinjections (100 and 150 ng/0.5 µl/hemisphere). The figure shows the average of maximum individual severity scores of dystonia reached within 1, 2 and 3 h after drug administration. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant improvement of dystonia in comparison to pre- *and* post-drug control (* $P<0.05$, ** $P<0.01$). Data are shown as means + S.E. of 8 (100 ng), 10 (150 ng), 11 (30 mg/kg) or 12 (20 mg/kg) mutant hamsters. For further explanation, see Fig. 2 legend.

dystonia (Table 1). The progression of dystonic attacks was delayed at a dose of 150 ng/hemisphere, while the lower dose of 100 ng did not reduce but even tended to increase the severity of dystonia (Fig. 4). All animals showed enhanced locomotor activity and ataxia from 5 to about 150 min after systemic treatments, while striatal injections of phenobarbital caused hypolocomotion during the first 60 min followed by enhanced motor activity.

In view of insufficient penetration into the brain, treatment with the endogenous ligand GABA was restricted to intrastriatal administrations. Striatal microinjections of GABA delayed the onset of dystonia in mutant hamsters at different life-periods (Table 1), but failed to exert significant effects on the severity of dystonia (Fig. 5; postmax: not illustrated). A dose of 200 µg/hemisphere caused a short-lasting (about 60 min), moderate reduction of spontaneous locomotor activity, while no behavioral effects could be observed at the lower doses of 50 and 100 µg.

At doses of 1.0, 1.5 and 2.5 mg/kg, i.p., the $GABA_A$ receptor antagonist bicuculline did not cause any significant effects on the severity of dystonia in groups of mutant hamsters at both ages (Fig. 6: postmax; max: not illustrated). Although striatal injections of 50, 100 and 150 ng/hemisphere caused a dose-dependent decrease of the latency to onset in dystonic hamsters which had passed the age of maximum severity (Table 1), a moderate increase of the severity of dystonia could be only observed at a dose of 100 ng (Fig. 6). As indicated by the early disappearance of adverse effects, i.e., a short-lasting hyperlocomotion and hyperexcitability after administration of 2.5 mg/kg, i.p., the duration of action was restricted to 30 min. At lower doses of 1 and 1.5 mg/kg, i.p., bicuculline did not provoke any behavioral effects. After striatal treatment with 50 and 100 ng/hemisphere bicuculline, the hamsters showed increased locomotor activity with interrupted forward movement, hyperexcitability and nodding. Furthermore, stereotypic oral movements and myoclonic jerks were provoked by the dose of 150 ng. These adverse effects lasted for about 30 min.

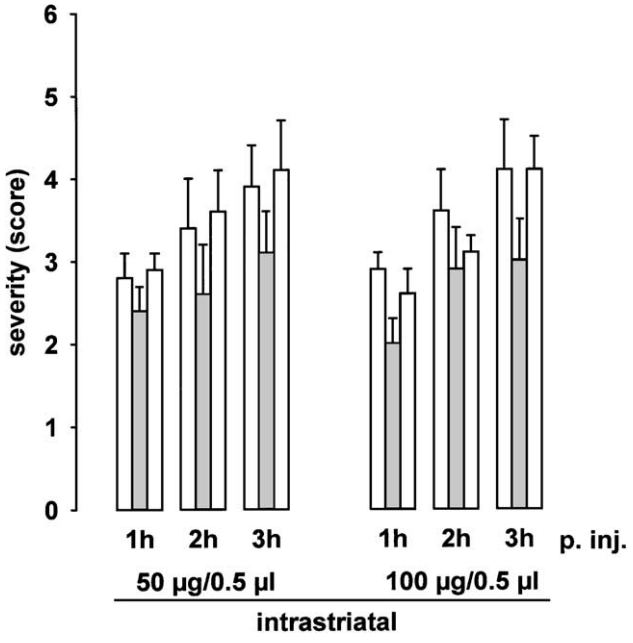

Fig. 5. Effect of GABA on the severity of dystonia in mutant hamsters after bilateral striatal injections of 50 and 100 µg/hemisphere. The figure shows the average of maximum individual severity scores of dystonia reached within 1, 2 and 3 h after administration of GABA. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Data are shown as means + S.E. of 8 dt^{sz} hamsters. For further explanation, see Fig. 2 legend.

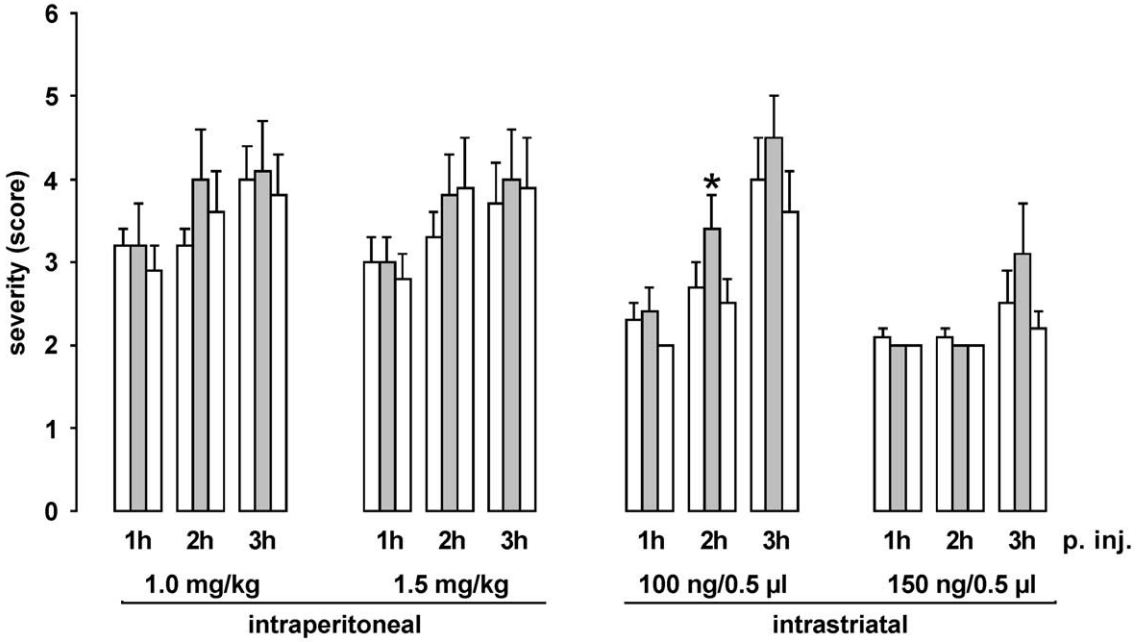

Fig. 6. Effect of the $GABA_A$ receptor antagonist bicuculline on the severity of dystonia in mutant hamsters after systemic administration (1.0 and 1.5 mg/kg, i.p.) and bilateral striatal microinjections (100 and 150 ng/0.5 µl/hemisphere). The figure shows the average of maximum individual severity scores of dystonia reached within 1, 2 and 3 h after the administration of bicuculline. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate a significant increase of the severity of dystonia in comparison to pre- *and* post-drug control (* $P < 0.05$). Data are shown as means + S.E. of 9 (1.5 mg kg/kg), 11 (150 ng), 12 (100 ng) or 13 (1.0 mg/kg) mutant hamsters. For further explanation, see Fig. 2 legend.

The antagonist of the benzodiazepine binding site flumazenil accelerated the progression of dystonia, i.e., increased the severity reached within the first hour (Fig. 7) and decreased the latency to onset of dystonia (Table 1) in mutant hamsters at age 50–60 days already at doses of 5 and 10 mg/kg, i.p. At a dose of 20 mg/kg i.p., flumazenil caused a significant increase of the maximum severity. Comparable effects were observed in younger animals (not illustrated). Striatal administrations of 100 ng/hemisphere caused a significant decrease of the latency to onset of dystonia (Table 1) and a significant increase of the severity of dystonia (2nd hour; Fig. 7), while 25 ng (not

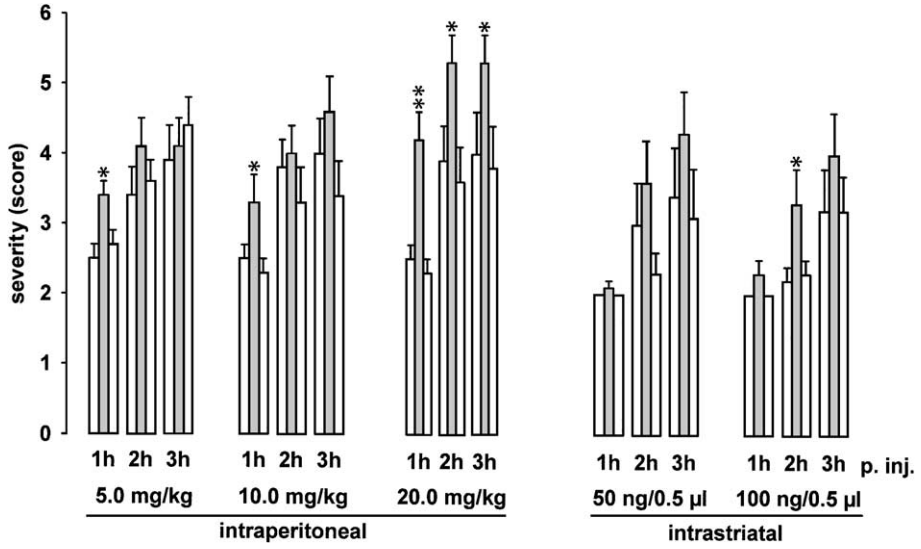

Fig. 7. Effect of the benzodiazepine receptor antagonist flumazenil on the severity of dystonia in mutant hamsters after systemic administration (5.0, 10.0 and 20.0 mg/kg, i.p.) and after bilateral striatal microinjections (50 and 100 ng/0.5 µl/hemisphere). The figure shows the average of maximum individual severity scores of dystonia reached within 1, 2 and 3 h after administration of flumazenil. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant aggravation of dystonia in comparison to pre- *and* post-drug control (* $P < 0.05$, ** $P < 0.01$). Data are shown as means + S.E. of 7 (50 ng), 10 (100 ng and 20 mg/kg), 11 (5 mg/kg) or 12 (10 mg/kg) dt^{sz} hamsters. For further explanation, see Fig. 2 legend.

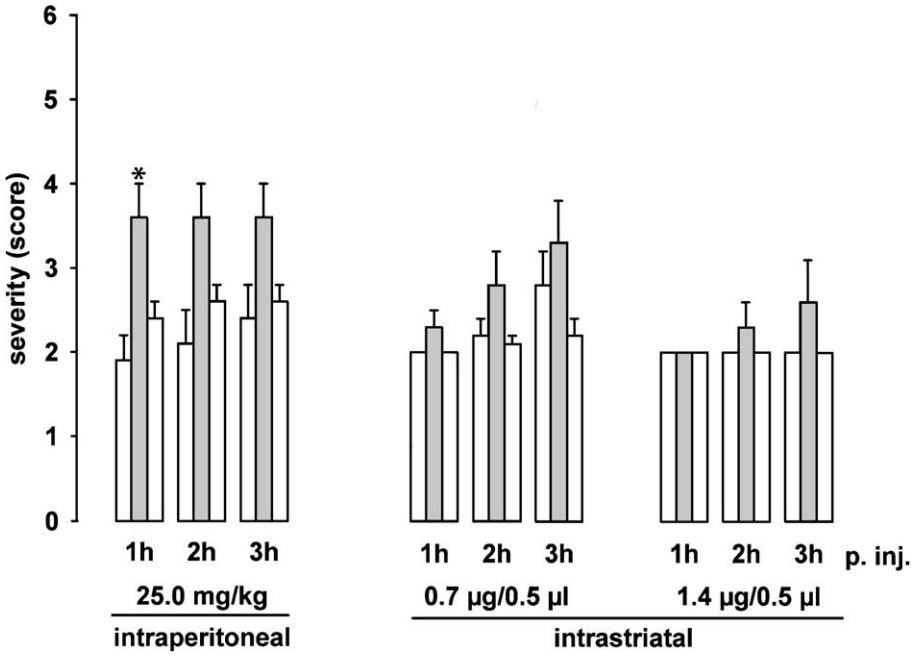

Fig. 8. Effect of pentylenetetrazole, which binds at the picrotoxinin site of the $GABA_A$ receptor complex, on the severity of dystonia in mutant hamsters after systemic administration (25.0 mg/kg, i.p.) and after bilateral striatal microinjections (0.7 and 1.4 μg/0.5 μl/hemisphere). The figure shows the average of maximum individual severity scores of dystonia reached within 1, 2 and 3 h after administration of pentylenetetrazole. Control recordings were taken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Asterisks indicate significant aggravation of dystonia in comparison to pre- *and* post-drug control ($*P<0.05$, $**P<0.01$). Data are shown as means + S.E. of 7 (25 mg/kg, postmax), 8 (1.4 μg) or 10 (0.7 μg and 25 mg/kg max) dt^{sz} hamsters. For further explanation, see Fig. 2 legend.

illustrated) or 50 ng did not exert any effects on the dystonic syndrome. Adverse effects were hyperlocomotion after systemic and striatal injections in dystonic animals (max and postmax). Furthermore, hyperexcitability could be observed in young mutant hamsters treated with higher doses of 20 mg/kg, i.p., or 100 ng/hemisphere. These side effects lasted 1–2 h, depending on the dose.

Pentylenetetrazole, which binds to the picrotoxinin site of the $GABA_A$ receptor complex and previously shown to aggravate dystonia in dt^{sz} mutants treated with 40 mg/kg, i.p. (Fredow and Löscher, 1991), increased the severity of dystonia already at a dose of 25 mg/kg, i.p. (Fig. 8; max: not illustrated) and accelerated the onset of dystonia (Table 1). Striatal injections of subconvulsive doses (0.7 and 1.4 μg/hemisphere) of pentylenetetrazole caused a significant decrease of the latency to onset of dystonic attacks (Table 1), but failed to exert significant effects on the severity (Fig. 8). Systemic and striatal administrations of pentylenetetrazole caused hyperlocomotion and hyperexcitability in mutant hamsters (max and postmax-period). These effects lasted 1–2 h.

4. Discussion

Previous extracellular single unit recordings have shown an overactivity of striatal GABAergic spiny projection neurons in anaesthetized dt^{sz} mutant hamsters (Gernert et al., 1999). Since the activity of these neurons is known to be inhibited by $GABA_A$ receptor activation (Niesenbaum and Berger, 1992), decreased GABA levels, a reduced expression of glutamate decarboxylase and a deficit of GABAergic parvalbumin-immunoreactive interneurons in the striatum of mutant hamsters (Burgunder et al., 1999; Gernert et al., 2000; Hörstermann and Löscher, 1992) indicated that striatal overactivity is based on GABAergic disinhibition. This is clearly supported by the antidystonic efficacy of the $GABA_A$ receptor agonist muscimol and of benzodiazepine flurazepam, shown in the present study after striatal microinjections. Furthermore, the effects of manipulating striatal benzodiazepine binding sites on the dystonic syndrome substantiate previous interpretations of abnormal [^{3}H]flumazenil binding in the striatum of dt^{sz} mutants; i.e., enhanced affinity and density of benzodiazepine binding sites obviously reflect a counteracting upregulation of these sites in response to a GABA deficit. After remission of dystonia in animals older than 10 weeks, flumazenil binding reached normal levels (Pratt et al., 1995), which might explain the age-dependent differences of behavioural effects observed in the present study after treatment with flurazepam. Although flumazenil is regarded as a specific benzodiazepine receptor antagonist, it should be noted that this compound exerts a slight partial agonistic activity under particular conditions, which is probably relevant for its anticonvulsant efficacy (Haefely, 1985). Since the agonistic action of flumazenil should dominate in the absence of

activation of benzodiazepine binding sites, the present finding of prodystonic effects of flumazenil together with the antidystonic activity of the full agonist flurazepam argue against the speculation that dystonia in mutant hamsters is due to a lack of endogenous benzodiazepines (Fisher and Iturrian, 1984; Richter and Löscher, 1998).

In contrast to muscimol and flurazepam, the barbiturate phenobarbital exerted only moderate antidystonic effects both after systemic and striatal administrations despite a long duration of action as indicated by the late disappearance of adverse effects. Benzodiazepines and barbiturates both increase the affinity of the $GABA_A$ receptor for GABA. Barbiturates, but not benzodiazepines, are also able to enhance the $GABA_A$ receptor-regulated chloride conductance in the absence of GABA at higher concentrations (Olsen, 1982; Sieghart, 1992). Phenobarbital also reduces by membrane-stabilizing effects the sodium conductance (MacDonald and McLean, 1986). Thus, the interneuronal network of striatal GABAergic parvalbumin-positive interneurons, which communicate through electrotonic coupling (Koos and Tepper, 1999), can be further disturbed in dt^{sz} hamsters. In view of the deficit of these neurons and evidence for altered neuronal synchronization in the striatum of mutant hamsters (Gernert et al., 1998, 2000) and pronounced prodystonic effects of sodium channel blockers (Richter et al., 1994), this mechanism of action may contribute to the lack of pronounced antidystonic effects after acute systemic and striatal injections of phenobarbital, observed in the present study, and its paradoxical prodystonic effects during chronic treatments in dt^{sz} hamsters, shown by previous examinations (Richter and Löscher, 2000).

Striatal GABAergic parvalbumin-reactive interneurons receive excitatory input from the cortex (Bennett and Bolam, 1994) and expression of dopamine D_2 receptors suggest that dopamine modulates their activity (Kawaguchi et al., 1995). As shown by Bevan et al. (1998), parvalbumin-positive interneurons are also innervated by pallidostriatal GABAergic neurons. Almost all of these interneurons express $GABA_B$ receptors (Yung et al., 1999). Since GABA activates both $GABA_A$ and $GABA_B$ receptors, the lack of significant effects on the severity of dystonia after striatal injections of GABA in mutant hamsters may in part be related to the inhibition of interneurons via $GABA_B$ receptors. Recent immunohistochemical examinations of $GABA_A$ receptor subunits in rats indicated that $GABA_A$ receptors are also located on GABAergic interneurons (Fujiyama et al., 2000). An increase of the activity of GABAergic interneurons by $GABA_A$ receptor blockade could be relevant for the lack of pronounced aggravation of dystonia in dt^{sz} mutants after striatal injections of compounds which reduce $GABA_A$ receptor-mediated inhibition.

Weak responses to striatal manipulations of GABAergic inhibition in mutant hamsters may in part be also based on a short action of the compounds, as indicated after systemic and striatal injections of bicuculline by the fast disappearance of behavioural effects. The rapid uptake of GABA into neurons and glia cells (Krogsgaard-Larsen et al., 1994) explains that there was only a delay in the onset of dystonia while severity was not reduced after striatal injections in dt^{sz} mutants. Furthermore, injections were restricted into the dorsal part of the striatum (caudate–putamen) where increased neuronal activity has been found by previous studies (Gernert et al., 1999; Richter et al., 1998). With regard to the recent findings of a significant reduction of the number and density of GABAergic interneurons within all striatal subregions in mutant hamsters (Gernert et al., 2000), disinhibition is, however, probably not restricted to the dorsal part. Quantification of GABAergic interneurons have as yet been restricted to the striatum. While most of the compounds examined in the present study exerted comparable effects on dystonia after striatal and systemic administrations, substantiating the importance of striatal GABAergic disinhibition in paroxysmal dystonia of dt^{sz} mutants, the effects of flurazepam and pentylenetetrazole appeared to be more marked after systemic than after striatal injections of doses which caused adverse effects after both routes of administration. These observations, together with previous findings of altered binding at the picrotoxinin binding site of $GABA_A$ receptor in dt^{sz} mutants (Nobrega et al., 1995), suggest that dysfunctions of the GABAergic system in other brain regions contribute to the dystonic syndrome. Thus, further studies have to clarify whether GABAergic interneurons are also reduced in ‘‘extrastriatal’’ structures.

In comparison to the present data on striatal manipulations of the $GABA_A$ receptor, recent microinjections of dopamine receptor agonists and antagonists into the dorsal striatum exerted in part more marked effects (Rehders et al., 2000). This does not necessarily argue against the suggestion that a deficiency of parvalbumin-reactive GABAergic interneurons in the striatum represents the primary defect in dystonic hamsters (Gernert et al., 2000), leading by disinhibition of GABAergic projection neurons to reduced basal ganglia output (Bennay et al., 2001; Gernert et al., 1999). However, in view of the paroxysmal nature of dystonia in dt^{sz} mutants, this permanent defect is obviously not sufficient to provoke dystonic episodes by itself. As indicated by the accelerated onset of dystonic attacks after striatal administration of bicuculline, flumazenil and pentylenetetrazole, and the delayed occurrence of dystonic symptoms after intrastriatal microinjections of GABA and GABA-potentiating drugs, the deficit possibly caused a disinhibition of stress-induced processes. Thus, dopaminergic overactivity, which is obviously essential for the manifestation of severe dystonia in mutant hamsters (Nobrega et al., 1996; Rehders et al., 2000) and possibly also in paroxysmal dystonia in humans (Todd and Perlmutter, 1998), could be secondary to the deficiency of GABAergic interneurons.

In summary, the fundamental role of disturbed GABAergic inhibition within the striatum in paroxymal dystonia of dt^{sz} mutants is supported by the present findings. The

present data underline the suggestion that considerations about the pathophysiology of dystonias should not be restricted to the dopaminergic system.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Ri 845/1-1). We thank Prof. Dr. W. Löscher for helpful discussions and Mrs. Ch. Bartling for her technical assistance.

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