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# Paradoxical aggravation of paroxysmal dystonia during chronic treatment with phenobarbital in a genetic rodent model

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**Abstract**

Recent studies in mutant hamsters ($dt^{sz}$), an animal model of primary paroxysmal dystonia, indicated that altered function of the γ-aminobutyric acid (GABA)ergic system plays a critical role in the pathogenesis of dystonia. In the present study, $dt^{sz}$ hamsters were chronically treated with phenobarbital, which has been found to exert antidystonic effects in mutant hamsters after acute administration. In untreated $dt^{sz}$ hamsters, the severity of dystonia follows an age-dependent time course with a maximum between the 30th and 40th day of life, followed by a continuous decline of severity until complete remission occurs at the age of about 70 days. In contrast to acute effects, chronic treatment with phenobarbital via drinking water starting at an age of 21 days (i.e., after weaning) worsened dystonia and retarded the spontaneous remission. The unexpected prodystonic effect was more marked after administration of higher doses and when chronic treatment with phenobarbital started at an age of 1 day (neonatal administration via breast milk). After withdrawal of phenobarbital at the age of 70 days, the severity rapidly declined in all treated groups. When phenobarbital was readministered 1 week later, the hamsters again exhibited severe dystonia. The mechanism of these unexpected findings is unknown. Tentatively, activity-dependent GABA-mediated excitation caused by chronic treatment with phenobarbital may be important for the prodystonic effects under pathological conditions in $dt^{sz}$ hamsters. 

*Keywords:* Movement disorder; Dystonia; Paroxysmal choreoathetosis; Barbiturate; GABA (γ-aminobutyric acid)

## 1. Introduction

The genetically dystonic hamster (gene symbol $dt^{sz}$) shows all phenotypic characteristics of human primary paroxysmal non-kinesiogenic dystonic choreoathetosis (paroxysmal dystonia), a type of dystonia in which attacks of dystonic and choreoathetotic movements precipitated by stress or caffeine last up to several hours (Demirkiran and Jankovic, 1995; Richter and Löscher, 1998). The pathophysiology of primary paroxysmal dystonia is unknown (Fahn, 1994; Demirkiran and Jankovic, 1995). In patients with paroxysmal dystonia, benzodiazepines (usually administered intermittently) have been reported to reduce the severity of this movement disorder (Fahn, 1994; Demirkiran and Jankovic, 1995). Data on neurochemical studies in patients with paroxysmal dystonia are not available, but the beneficial effects of benzodiazepines could suggest that disturbed γ-aminobutyric acid (GABA)ergic inhibition is involved in the pathogenesis of paroxysmal dystonia (Demirkiran and Jankovic, 1995).

In $dt^{sz}$ hamsters, the severity of dystonic attacks, which can be induced by mild stress, such as handling or change in environments (Löscher et al., 1989), shows an age-dependent time course with the first occurrence of dystonia at about 16 days. The animals show maximum severity of dystonia at the day of weaning (21st day) and between 30 and 40 days of life. Thereafter, the severity slowly declines until complete remission of dystonia occurs at the age of about 60–70 days (Richter and Löscher, 1993; Löscher et al., 1995). Several findings from pharmacological and neurochemical studies have indicated that dysfunctions of the GABAergic system are critically involved in the dystonic syndrome in $dt^{sz}$ hamsters (Richter and Löscher, 1998). Acute treatment with GABA-potentiating drugs, such as the benzodiazepine diazepam and the barbiturate phenobarbital were found to exert antidystonic effects in mutant hamsters, while compounds that impair $GABA_A$ receptor-mediated inhibition, such as the inverse benzodiazepine receptor agonist *N*-methyl-β-carboline-3-carboxamide (FG 7142) and pentylentetrazole, which re-

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duces $GABA_A$ receptor function by binding to the picrotoxinin site of the $GABA_A$ receptor complex, increased the severity of dystonia (Fisher and Iturrian, 1984; Fredow and Löscher, 1991). Neither FG 7142 nor pentylentetrazole induced motor effects in non-dystonic hamsters at doses that were prodystonic in mutant hamsters (Fredow and Löscher, 1991). The antidystonic efficacy of GABA-potentiating drugs (diazepam, phenobarbital and aminooxyacetic acid) was found to be age-dependent, i.e., more marked at an age of 31 days than 21 days, while no age dependence became evident for pharmacological sensitivity of dopaminergic and glutamatergic systems in *dt*$^{sz}$ hamsters (Richter and Löscher, 1993). This finding could point to a transient functional subsensitivity of the GABAergic system due to retarded development of GABAergic inhibition.

Neurochemical examinations in mutant hamsters revealed changes of the GABAergic system particularly within the striatum. With regard to pharmacological observations, lower GABA levels and glutamic acid decarboxylase mRNA in the striatum of mutant hamsters (Richter and Löscher, 1998), enhanced affinity and density of benzodiazepine binding sites in the striatum were interpreted as an upregulation of benzodiazepine receptors. Changes of benzodiazepine binding disappeared in parallel to the spontaneous remission of stress-inducible dystonia (Pratt et al., 1995). Nobrega et al. (1995) found an increased binding of [$^{35}$S]*t*-butylbicyclophosphorothionate (TBPS) to the picrotoxinin binding sites of the $GABA_A$ receptor complex in several brain regions of mutant hamsters. Since GABA-potentiating drugs, such as benzodiazepines and barbiturates, allosterically inhibit [$^{35}$S]TBPS binding, while convulsant β-carbolines, such as FG7142, enhance the binding by interaction with the benzodiazepine site (Sieghart, 1992), the abnormal [$^{35}$S]TBPS binding in mutant hamsters is consistent with pharmacological findings after acute treatment.

Apart from neonatal treatment with diazepam (Fisher, 1986), previous pharmacological examinations in mutant hamsters were restricted to acute administrations of drugs, such as phenobarbital (Richter and Löscher, 1998), although dystonias usually deserve chronic medication in humans (Fahn, 1995). Therefore, mutant hamsters were chronically treated in the present study. With regard to recent findings, which indicated that dysfunctions of $GABA_A$ receptor play a critical role in the pathogenesis of dystonia in *dt*$^{sz}$ hamsters (Richter and Löscher, 1998), for these investigations, phenobarbital was chosen because this barbiturate reduced the severity of dystonia in mutant hamsters after acute administration of 20 mg/kg i.p. (Fredow and Löscher, 1991) and the water solubility of the sodium salt of phenobarbital allowed administration via drinking water from day 21 on (age of weaning). In order to examine if early treatment with phenobarbital can prevent the development of age-dependent dystonia in mutant hamsters, one experimental group was treated neonatally via breast milk.

## 2. Materials and methods

### 2.1. Animals

The experiments were carried out in groups of male and female mutant dystonic hamsters (genetic symbol *dt*$^{sz}$). The hamsters were obtained by selective breeding (for detailed description, see Fredow and Löscher, 1991; Richter and Löscher, 1998). After weaning at an age of 21 days, all hamsters were housed in groups of five to eight animals in Makrolon cages at constant temperature (21–23°C) and 12-h light cycle (light on at 0700 h). The animals had ad libitum access to standard diet and water (control group) or drug solution (phenobarbital treated groups). All behavioural observations were carried out between 0900 and 1200 h at controlled temperature.

### 2.2. Behavioural testing

Dystonic attacks in *dt*$^{sz}$ mutant hamsters, characterized by generalized dystonic and choreoathetotic movements, can be induced by handling and mild environmental stimuli. In the present pharmacological examinations, the dystonic attacks were induced every second to third day after weaning by the procedure of triple stimulation (Löscher et al., 1989; Richter and Löscher, 1998), i.e., (1) taking the animal from its home cage and placing it on a balance, (2) i.p. injection of saline, and (3) placement of the hamster in a clean and empty plastic cage (one animal per cage). The severity of dystonia was rated by following score system (Löscher et al., 1989): stage 1, flattened ears and flattened posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with retarded setting of the forepaws; stage 3, stiffened hindlimbs so that the animals appear to walk on tiptoes in a dysmetric hypergait; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; and stage 6, immobilisation in a twisted, hunched posture with hind- and forelimbs tonically extended forward, Straub tail, alternating unilateral forelimb elevation, opisthotonus, copious red eye mucus and salivation. After reaching the individual maximum stage the hamsters usually 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 hamsters were observed for 3 h. During this period, the severity of dystonia, the latencies to the different stages and in the treated groups the side effects were noticed. In all experimental groups (see below), the age-dependent time course of dystonia was determined by testing the animals every 2–3 days over a period of 6–9 weeks. During the 3-h observation period (from 0900 to 1200 h), the animals did not receive drinking water (control) or phenobarbital solution (treated groups).

### 2.3. Drug administration

In groups of 9–19 male and female *dt*$^{sz}$ mutant hamsters, phenobarbital was administered via drinking water in

order to allow continuous drug intake without induction of dystonic attacks by injections several times a day. The concentrations of phenobarbital per milliliter of drinking water chosen in the present study are based on a series of preceding experiments (not illustrated), including the registration of the daily water intake (mean consumption: 92 ml/kg body weight/day; range 70–130 ml/kg) and pharmacokinetic examinations in hamsters, such as determinations of the plasma half-life time of phenobarbital (6 h after acute i.p. injection of the acute antidystonic effective dose of 20 mg/kg), of the oral bioavailability (94%) and of the acute anti-dystonic effective plasma level (20–40 μg/ml plasma).

As previously shown by Frey and Kampmann (1965), the acute anticonvulsant effective dose of phenobarbital (30 mg/kg/day p.o.) had to be increased in mice during chronic treatment for 2 weeks via drinking water to 100 mg/kg/day in order to counterbalance the development of metabolic and functional tolerance. With regard to a comparable half-life time of phenobarbital in mice (6.5 h; Frey and Kampmann, 1965) and hamsters (6 h) and similar acute toxic doses ($LD_{50}$ p.o.) in mice (280 mg/kg; Gattermann and Fiedler, 1983) and hamsters (270 mg/kg; Vida and Gerry, 1977), a threefold increase of the initially effective dose was considered to be also suitable for chronic experiments in hamsters. This was proven in a series of preceding experiments. In brief, the development of metabolic tolerance was examined in two groups of six non-dystonic Syrian hamsters, which received 0.5 or 1.0 mg phenobarbital/ml drinking water for 1 week. The animals were single housed, allowing determinations of individual drug intake. Depending on the consumption of drug solution, the daily drug intake (including loss of drug solution by dropping nipple-bottles directly after drinking) was 33–124 mg/kg/day in animals, which received 0.5 mg/ml/day and 46–232 mg/kg/day in hamsters treated with 1.0 mg/ml phenobarbital solution. In both groups, metabolic tolerance became evident by a decrease of the half-life time to 2–3 h within the first 3 days of treatment. Nevertheless, an intake of 0.5 mg/ml during the first 24 h led to acute effective plasma levels (30–69 μg/ml plasma; $55.2 \pm 6.2$). After a 3-h deprivation of phenobarbital solution (necessary for the 3-h observation period as described above), acute effective plasma levels of 20 μg/ml were still present ($27.3 \pm 2.8$ μg/ml). Therefore, chronic experiments were started with a phenobarbital concentration of 0.5 mg/ml drinking water. Development of tolerance and variation of individual drug intake required an increase of the drug concentration in drinking water. Even at a drug concentration of 1.0 mg/ml, subeffective plasma levels were observed at the fourth day of treatment. For this reason, the drug concentration was increased during the first week of chronic treatment up to 1.75 mg/kg as described below. In the preceding experiments, the applicability of the chosen drug concentrations were tested in a group of 12 non-dystonic Syrian hamsters, including determinations of plasma concentrations and observations of sedation and of ataxia by the rotorod-test. Marked sedation and ataxia were caused after an increase of the drug solution to 1.75 mg/ml, while only moderate adverse effects were observed with lower concentrations, such as 1.3 mg/ml. However, within 3 days, the adverse effects decreased, probably based on functional tolerance. Phenobarbital did not cause a reduction of body weight. High concentrations of phenobarbital in the drinking water did not reduce the consumption of solution. After termination of phenobarbital treatment no withdrawal symptoms could be observed. One group of dystonic hamsters (Ia; see below) received, therefore, this high concentration of 1.75 mg/ml phenobarbital in order to make sure that effective plasma levels were maintained over the whole period of treatment, while in the other groups (Ib, II) a maximum concentration of 1.3 mg/ml was administered in order to avoid toxic doses.

Phenobarbital, used as sodium salt, was provided by Desitin Arzneimittel (Hamburg, FRG). The phenobarbital solutions were freshly prepared every day. The bottles containing phenobarbital solution were weighed every day in order to calculate the daily drug intake in milligrams per kilogram body weight. The body weight of the animals was recorded every day of testing as described above.

### 2.4. Chronic treatment of the experimental groups

In two groups of $dt^{sz}$ hamsters, chronic treatment with phenobarbital started after the first induction of a dystonic attack on the day of weaning (at 21 days of life). To counterbalance the development of tolerance and to maintain higher than acute anti-dystonic effective plasma levels (see above) over the whole period of treatment, the initial drug concentration of 0.5 mg phenobarbital per milliliter of drinking water had to be increased during the first days of treatment (age 22 days: 0.7 mg; age 23 days: 1.0 mg; age 24 days: 1.3 mg; age 26: 1,5 mg/ml). One group (group Ia) received a drug concentration of 1.75 mg/ml from the 28th day on. The other group (group Ib) got a maximum concentration of 1.3 mg/ml from the age of 24 days on. The drug concentrations of 1.75 mg (group Ia) or 1.3 mg/ml (group Ib) were continuously administered until an age of 69 days.

Furthermore, one group (group II) was treated neonatally (during days 1.0–21.0 of life intake of phenobarbital via breast milk). Therefore, the dams received 0.5–2.0 mg phenobarbital per milliliter of drinking water during the period of nursing: 0.5 mg on day 1 post natum, 0.7 mg on day 2, 1.0 mg on day 3, 1.3 mg on days 5 and 6, 1.5 mg on days 7 and 8, 1.75 mg from days 9 to 12, and 2.0 mg/kg phenobarbital from days 13 to 21 post natum. After weaning, the litters were treated like group Ib.

At an age of 69 days, phenobarbital was withdrawn in all treated groups for 7 days and then phenobarbital solutions of 1.3 mg/ml (groups Ib and II) or 1.75 mg/kg (group Ia) were readministered for 1 week. As described

above, in all treated groups and an untreated group (control) of *dt*$^{sz}$ hamsters, supplied with normal drinking water, dystonic attacks were induced every 2–3 days in order to compare the severity of dystonia between treated and untreated groups.

### 2.5. Determinations of phenobarbital plasma levels

Apart from determinations of phenobarbital plasma levels in preceding experiments in which the pharmacokinetic characteristics were examined in hamsters (see above), the concentrations were determined weekly in the chronic experiments, i.e., in six mutant hamsters of group I and group II. Therefore, blood was sampled (about 0.5 ml) by orbital puncture 1 day after behavioural testing at 0900 h and after a 3-h deprivation period of phenobarbital at 1200 h. The blood samples were immediately centrifuged, and plasma was stored at −20°C until analyses. Phenobarbital determinations in plasma were done by gas chromatography as described elsewhere (Löscher and Göbel, 1978).

### 2.6. Statistics

All data are shown as means + S.E. The individual age-dependent time course of dystonia was characterized for each animal by determining the area under the curve (AUC) of severity as a function of age (age: 21–69 days). For the drug intake over the whole period of treatment, the AUC of the mean daily doses was calculated. The significance of differences between the AUCs of the control and phenobarbital-treated groups was calculated by Student's *t*-test.

## 3. Results

In untreated *dt*$^{sz}$ mutant hamsters (control), the severity of dystonia slowly declined after a period of maximum severity scores between the 30 and 40 days of life (Fig. 1). With the age of 66 days, the animals had completely lost their susceptibility to induction of dystonia by stressful stimuli. The AUC under the severity/time curve between the 21st and 68th day was 152 ± 7 score day (Table 1). As reported previously (Löscher et al., 1995), there was no difference in the time course of dystonia between male and female mutant hamsters.

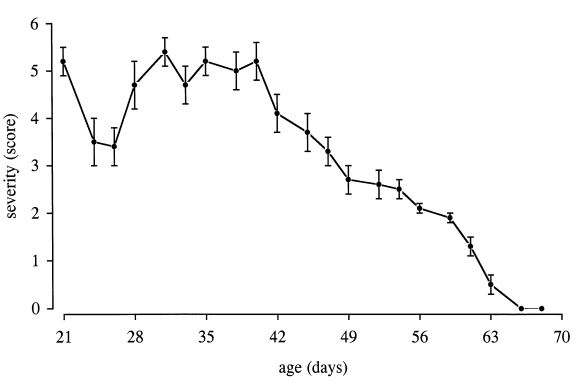


Fig. 1. Age-dependent time course of the severity (score) of dystonia in untreated *dt*$^{sz}$ mutant hamsters (control group). Dystonic attacks were induced every 2–3 days by stressful stimuli from the age of 21 days (day of weaning) until the animals had completely lost their susceptibility of induction of dystonia by stress. Severity of a dystonic attack was scored by a severity rating scale during a 3-h observation period, and the individual maximum severity reached in this period was used for calculation of average group values. The maximum severity of the attacks is shown as the mean ± S.E. of 15 dystonic hamsters.

Table 1
Means ± S.E. of the AUC of the age-dependent time course of the severity of dystonia between the 21 and 68 days of life in untreated *dt*$^{sz}$ mutant hamsters (control) and groups of mutant hamsters, which were chronically treated with phenobarbital (Ia, Ib, II). As indicated by AUC of the average daily drug intake between the 21 and 68 days of life, group Ia received a higher dose than groups I b and II. Group II was neonatally treated with phenobarbital before the day of weaning (21 days of age) via breast milk (not considered in the AUC of daily drug intake). Significant differences of the AUC of severity of dystonia ($P < 0.001$) between control (c) and treated groups as well as significant differences between treated groups are indicated in the table

| Group | Number of animals | AUC of average drug intake (mg day) | AUC of severity of dystonia (score day) | $P < 0.001$ compared to |
|---|---|---|---|---|
| Control (c) | 15 | 0 | 152 ± 7 | |
| Ia | 9 | 9386 | 225 ± 6 | c and Ib |
| Ib | 19 | 5586 | 186 ± 4 | c |
| II | 18 | 6328 | 226 ± 6 | c and Ib |

As shown in Fig. 2, in mutant dystonic hamsters chronically treated with high concentrations of phenobarbital in the drinking water (group Ia), resulting in a dose of 50–300 mg/kg body weight/day (AUC of phenobarbital intake from 21 to 68 days: 9386 mg day; Table 1), the spontaneous remission of dystonia was clearly delayed in comparison to the control group. The AUC of severity of dystonia (225 ± 6 score day) was significantly higher than in untreated hamsters ($P < 0.001$). The incidence of spontaneous dystonic attacks was increased, i.e., the *dt*$^{sz}$ hamsters often exhibited already severe dystonia before stimulation procedure. During the first 2 weeks of treatment, phenobarbital caused marked ataxia and sedation (not illustrated). Thereafter, the adverse effects were moderate or absent, probably due to pharmacodynamic tolerance because high plasma levels were maintained during the whole period of treatment (see Table 2). Some older animals showed hyperlocomotion. After a deprivation of phenobarbital for 3 h during the period of observation (1200 h), the plasma levels were still higher than the acutely effective plasma levels (20–36 μg/ml). The fluctuation of daily drug intake was higher than in preceding experiments and led to variable plasma levels. When phenobarbital was withdrawn at an age of 68 days, a rapid decline of the severity of dystonia became evident (Fig. 2). When phenobarbital was readministered after a drug-free interval of one week, the hamsters again exhibited severe dystonic

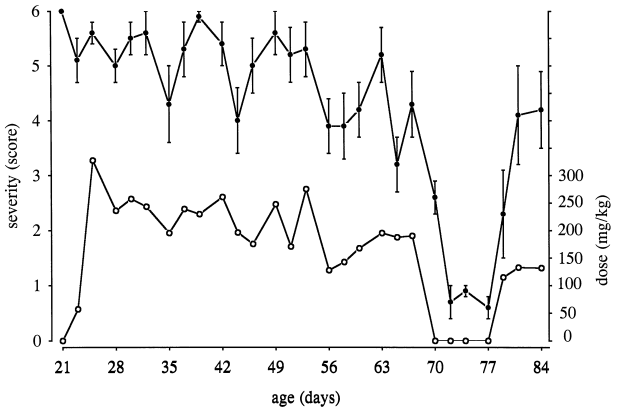


Fig. 2. Age-dependent time course of dystonia in a group of nine $dt^{sz}$ mutant hamsters (group Ia), which received high doses of phenobarbital via drinking water from day 21 (treatment started after the initial recording of dystonia) to day 68. The dystonia severity scores are shown as the means ± S.E. (filled circles), the average of daily intake of phenobarbital is shown by open circles. Note that the severity of dystonia rapidly declined during a drug-free interval between an age of 70 and 77 days. For further explanations, see Fig. 1 legend.

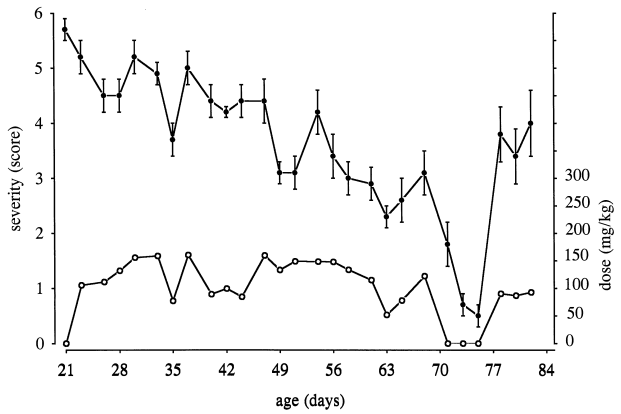


Fig. 3. Age-dependent time course of dystonia in a group of 19 $dt^{sz}$ mutant hamsters (group Ib), which received lower doses of phenobarbital via drinking water from day 21 (treatment started after the initial recording of dystonia) to day 68. The dystonia severity scores are shown as the means ± S.E. (filled circles), the average of daily intake of phenobarbital is shown by open circles. For further explanations, see Fig. 2 legend.

attacks. Furthermore, the animals showed locomotor hyperactivity.

Although chronic treatment with lower phenobarbital concentrations (1.3 mg/ml drinking water) also significantly increased the severity of dystonia in group Ib in comparison to the control group (AUC 186 ± 4 score day, $P < 0.001$, Table 1), a tendency of an age-dependent remission of dystonia could be observed (Fig. 3). In animals of group Ib in which the daily drug intake varied between 50 and 160 mg/kg (Fig. 3) during the period of administration of phenobarbital from the 21 to 68 days of life (AUC 5586 mg day) the aggravation of dystonia was significantly less marked than in group Ia ($P < 0.001$). In contrast to group Ia, the hamsters of group Ib showed only moderate adverse effects (ataxia and sedation) during the first week of treatment. Thereafter, no side effects could be observed. As shown in Table 2, the acutely effective plasma concentration was exceeded over the whole period of treatment (usually also after a 3-h deprivation of phenobarbital). Similar to group Ia, a rapid reduction of the severity of dystonia could be observed after withdrawal of phenobarbital at an age of 68 days (Fig. 3). After readministration of phenobarbital, the severity rapidly increased.

Fig. 4 shows the age-dependent time course of dystonia in mutant dystonic hamsters of group II (three litters), which were already treated via breast milk from the first day of life on. Therefore, the dams ($n = 3$) of these litters received phenobarbital during the whole period of nursing via drinking water, resulting in a mean drug intake of the dams of 18–77 mg/kg/day from day 1 to 12 of nursing and of 149–305 mg/kg/day from day 13 to 21 of nursing. After weaning on day 21, group II was treated like group Ib. Similar to groups Ia and Ib, the animals of group II showed a delay of remission of dystonia (Fig. 4) and a

Table 2
Phenobarbital plasma levels (μg/ml) determined weekly in six mutant hamsters of group I and in six hamsters of group II during the period of treatment. The data are shown as the range at 0900 h and after 3 h deprivation of phenobarbital at 1200 h

| Week of treatment | Group Ia | | Group Ib | | Group II | |
|---|---|---|---|---|---|---|
| | 0900 h | 1200 h | 0900 h | 1200 h | 0900 h | 1200 h |
| 1 | 64–104 | 48–76 | 43–71 | 28–46 | 40–69 | 29–45 |
| 2 | 72–110 | 50–81 | 76–136 | 35–106 | 74–144 | 35–85 |
| 3 | 192–210 | 109–170 | 86–111 | 65–85 | 43–89 | 26–57 |
| 4 | 48–61 | 26–39 | 56–77 | 37–51 | 53–94 | 24–47 |
| 5 | 100–177 | 66–127 | 58–92 | 26–59 | 36–86 | 22–49 |
| 6 | 90–94 | 65–67 | 35–104 | 18–76 | 46–96 | 27–56 |
| 7 | 85–139 | 53–85 | 83–137 | 50–87 | 26–59 | 13–45 |

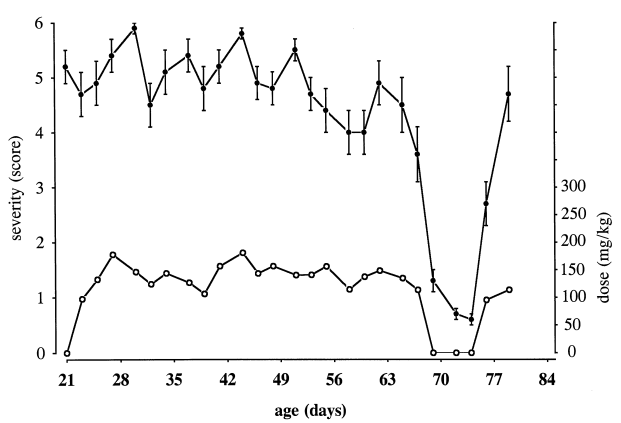


Fig. 4. Age-dependent time course of dystonia in a group of 18 $dt^{sz}$ mutant hamsters (group II), which was neonatally treated with phenobarbital via breast milk until day of weaning (21 days). From day 21 to day 68, the animals received phenobarbital via drinking water as group Ib. The dystonia severity scores are shown as means ± S.E. (filled circles), the daily intake of phenobarbital is shown by open circles. For further explanations, see Fig. 2 legend.

significant increase of the severity of dystonic attacks in comparison to the control group (Table 1). During the post-weaning period, the daily doses of phenobarbital reached 97–177 mg/kg (Fig. 4) and the AUC of drug intake was 6328 mg day (Table 1). Although the doses during this period were comparable to those of group Ib, the neonatally treated hamsters showed significantly higher severity of dystonia than group Ib ($P < 0.001$, Table 1). At an age of 7 weeks for example, group Ib showed a mean severity score of $3.1 \pm 0.2$ after intake of 134 mg/kg/day, while in hamsters of group II, a score of $4.9 \pm 0.3$ was reached after a drug intake of 140 mg/kg. Apart from enhanced locomotor activity in some hamsters, no side effects were observed during the post-weaning period in group II. As shown in Table 2, the acutely effective plasma concentrations of phenobarbital were exceeded during the post-weaning period before and usually also after a 3-h deprivation of phenobarbital. Similar to the observations in groups Ia and Ib, the severity of dystonia rapidly declined after withdrawal of phenobarbital at an age of 68 days, and readministration of phenobarbital aggravated the dystonic syndrome (Fig. 4).

As observed in preceding experiments, phenobarbital did not reduce the body weight or consumption of drinking water in all experimental groups. Since dystonia was aggravated in the absence of marked sedation or ataxia (not illustrated), the prodystonic effects were obviously not due to toxicity of phenobarbital.

## 4. Discussion

In line with recent observations (Löscher et al., 1989, 1995; Richter and Löscher, 1993), untreated mutant hamsters of the present study showed a characteristic age-dependent time course of dystonia with spontaneous remission at an age of about 10 weeks. However, there are also observations of relapses of dystonic attacks in pregnant $dt^{sz}$ hamsters (Khalifa and Iturrian, 1993) and drug-provoked dystonic attacks in animals, which had lost the susceptibility of stress-inducible dystonia (Richter et al., 1997), which argue against a transient type of paroxysmal dystonia as described in children (Angelini et al., 1988).

The prodystonic effect of phenobarbital during chronic treatment shown by the present data was an unexpected finding because phenobarbital exerted antidystonic effects after acute administration (Fredow and Löscher, 1991; Richter and Löscher, 1993). In all groups of $dt^{sz}$ hamsters chronically treated with phenobarbital, a notable delay of the age-dependent regression of dystonia was found in comparison to untreated $dt^{sz}$ hamsters. The aggravation of dystonia was more marked when the hamsters received higher doses (group Ia vs. group Ib) and when the hamsters were earlier treated with phenobarbital (group II vs. group Ib). The prodystonic effects during chronic treatment cannot be explained by tolerance and drug dependence because effective plasma concentrations of phenobarbital were maintained during the treatment period and the severity of dystonia rapidly decreased after withdrawal of phenobarbital. Since dystonia was worsened already 1 day after readministration of phenobarbital, the prodystonic effect of phenobarbital seems to be related to fast biochemical changes, which probably disturb mechanisms that lead to disappearance of dystonia in mutant hamsters.

Opposite acute and chronic effects of the GABA-potentiating drugs phenobarbital and sodium valproate have been also observed in a strain of Mongolian gerbils, which is predisposed to spontaneous seizures (Watanabe et al., 1978; Cutler and Horton, 1988). While phenobarbital or valproate are effective anticonvulsant agents in seizure-sensitive gerbils after acute administration, chronic treatment in developing gerbils caused an intensification of seizure activity. In contrast to the prodystonic effects in mutant hamsters, the enhancement of seizure activity persisted long after termination of drug treatment (Watanabe et al., 1978; Cutler and Horton, 1988). The long-lasting proconvulsant effect of valproate was not due to changes in saturation binding to the binding sites of GABA, benzodiazepines, picrotoxinin (Cutler and Horton, 1988). Although the $dt^{sz}$ hamster does not represent a model of epilepsy, but of paroxysmal dystonia as demonstrated by detailed examinations (Richter and Löscher, 1998), in both epileptic gerbils and dystonic hamsters, dysfunctions of the GABA receptor complex have been suggested to play a critical role for the neurological symptoms (Löscher, 1985; Olsen et al., 1985; Richter and Löscher, 1998). While epileptic gerbils exhibit permanent seizure susceptibility, possibly due to a deficit of the $GABA_A$ receptor complex in the midbrain (Olsen et al., 1985), $dt^{sz}$ hamsters show an age-dependent remission of dystonia accompanied by disappearance of neurochemical changes of the GABAergic system (Pratt et al., 1995; Richter and Löscher, 1998). This different pathological condition could be relevant for the rapid decline of severity of dystonia observed in mutant hamsters after termination of chronic treatment with phenobarbital in contrast to animal models of epilepsy.

Neurochemical changes of the GABAergic system were particularly detected in the striatum of $dt^{sz}$ hamsters (Richter and Löscher, 1998). Chronic administration of phenobarbital was found to lead to decreased $GABA_A$ receptor binding in the striatum, but not in other brain regions of rats (Möhler et al., 1978), while other authors reported a receptor down-regulation in several brain regions (Liljequist et al., 1984; Fares et al., 1990). Thus, an inherited deficit of GABAergic inhibition in mutant hamsters would be worsened by chronic treatment with phenobarbital, leading to aggravation of dystonia particularly after drug withdrawal. However, the severity of dystonia rapidly declined after termination of phenobarbital treatment. Since barbiturates are able to enhance $Cl^-$ conductance in the absence of GABA, particularly at higher concentrations (Macdonald and Olsen, 1994; Möhler,

1992), the more marked aggravation of dystonia by higher doses of phenobarbital let us conclude that the present data represent paradoxical drug effects.

Paradoxical effects of phenobarbital and other GABA-potentiating drugs have been described in humans and rodents (Diaz and Schain, 1977; Watanabe et al., 1978; Cutler and Horton, 1988; Lanius et al., 1993). Although the mechanism underlying paradoxical drug action is still unknown, there is evidence for an age-related component to these observations (Lanius et al., 1993). As shown in the present examinations, the delay of remission of dystonia was more marked when the hamsters were treated neonatally, indicating developmental abnormalities of GABAergic inhibition. In early postnatal life, the inhibitory neurotransmitter GABA can depolarize and excite neuronal membranes (Cherubini et al., 1991), possibly related to the subunit composition of $GABA_A$ receptors (Olsen and Avoli, 1997), which is regulated by $GABA_A$ receptor stimulation during development (Poulter et al., 1997). There is evidence that $GABA_A$ receptor-mediated depolarization in neonatal neurons is due to enhanced efflux of bicarbonate ions via the GABA-activated ionophore (Cherubini et al., 1991). Excitation mediated by intensely activated $GABA_A$ receptors can be enhanced by barbiturates (Staley, 1992). This mechanism could explain the paradoxical aggravation of dystonia and the unexpected observation of locomotor hyperactivity during chronic treatment with phenobarbital in mutant hamsters. A pathophysiologically reduced GABAergic inhibition, as indicated by recent studies (Richter and Löscher, 1998), would be normalized by acute treatment with GABA-potentiating drugs (Fredow and Löscher, 1991), while chronic overstimulation of $GABA_A$ receptors by phenobarbital could further disturb GABAergic inhibition in a dose-dependent manner as indicated by the present study. An abnormal anionic gradient shift via the $GABA_A$ receptor-regulated ionophore may be more marked in neonatal neurons (Cherubini et al., 1991; Olsen and Avoli, 1997; Rivera et al., 1999), which could therefore explain the more marked aggravation of dystonia in neonatally treated hamsters. Since the activity-dependent collapse of the opposing concentration gradients of $Cl^-$ and bicarbonate ions probably underlies a rapid normalization or development, the fast decline of dystonia after withdrawal and a fast reoccurrence of severe dystonia after readministration of phenobarbital in $dt^{sz}$ hamsters would be understandable. The GABA-mediated activity-dependent depolarization under the pathological condition could be also important for human paroxysmal dystonia because acetazolamide exerts beneficial effects (Fahn, 1994). Acetazolamide, which blocks the bicarbonate regeneration, can reduce GABA-mediating excitation without affecting GABA-mediated inhibition (Staley et al., 1995).

Although the mechanism of the present finding remains unknown, the data support the suggestion of Fouad et al. (1996) that the $GABA_A$ receptor is a good candidate for the site of defect in paroxysmal dystonia and confirm recent neurochemical findings in mutant hamsters, which indicated that dysfunctions of the GABAergic system play a crucial role in the pathogenesis of paroxysmal dystonia (Richter and Löscher, 1998).

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