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# Inhibition of Pentylenetetrazol Induced Genetically-Determined Stereotypic Convulsions in Tottering Mutant Mice by Diazepam

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SYAPIN, P J *Inhibition of pentylenetetrazol induced genetically-determined stereotypic convulsions in tottering mutant mice by diazepam* PHARMACOL BIOCHEM BEHAV **18**(3) 389–394, 1983 —Genetically-determined stereotypic behavioral convulsions (GDSC) resulting from the autosomal recessive mutation tottering *(tg)* were studied in C57BL/6J mice GDSC was found to be reliably induced in *(tg/tg)* homozygotes with 30 mg/kg pentylenetetrazol (PTZ), a dose that is significantly below the threshold for induction of generalized clonic convulsions in *(tg/+)* heterozygotes and (+/+) wild type C57BL/6J mice The effects of anticonvulsant drugs on GDSC were studied in *(tg/tg)* mice induced by 30 mg/kg PTZ Ethosuximide (250 mg/kg), phenytoin (25 and 50 mg/kg), sodium phenobarbital (25 and 50 mg/kg), naloxone (6 mg/kg), valproic acid (150 mg/kg), sodium valproate (200 mg/kg) and aminooxyacetic acid (25 mg/kg) were all without effect on PTZ induced GDSC In contrast to its usual anticonvulsant action, phenytoin caused GDSC in *(tg/tg)* mice Diazepam (4 mg/kg) was completely effective in blocking GDSC 2 hr post administration Ethosuximide (250 mg/kg), which did not protect *(tg/tg)* mice against GDSC was able to protect both *(tg/tg)* and *(tg/+)* mice against generalized clonic-tonic convulsions induced by 85 mg/kg PTZ These results suggest a novel mechanism of action for the induction of GDSC by 30 mg/kg PTZ in *(tg/tg)* mice which may involve benzodiazepine receptors

Tottering mouse Genetic mutation Convulsions Epilepsy Pentylenetetrazol Diazepam Phenytoin

MICE homozygous for the genetic mutation named tottering (gene designation *tg*, chromosome 8, autosomal recessive) are phenotypically distinguished by a hindlimb ataxia and "spontaneous" genetically-determined stereotypic behavioral convulsions (GDSC) i e , repeatedly occurring, compulsive sets of reproducible abnormal motor activities appearing the same in all *(tg/tg)* mice (see [4] for detailed description of the GDSC) The spontaneous-convulsing phenotype is not identified until 4–8 weeks postnatal, depending on the mouse strain used, and in other respects appears normal, they are fertile, attain normal body weight as adults, and seem to have normal longevity

The discovery of the *tg* mutation and its subsequent transfer to the highly inbred C57BL/6J strain provides a stable, homogeneous population to study how a genetic trait can influence the susceptibility of the nervous system towards naturally occurring seizures and convulsions However, knowledge about the tottering mouse is limited The cellular etiology of the spontaneous-convulsing phenotype is presently unknown The brain of the C57BL/6J *(tg/tg)* mouse appears morphologically normal ([12] and unpublished observations) although differences in the density of innervation by catecholamine-containing neurons of the locus ceruleus have been reported between *(tg/tg)* and wild type (+/+) mice [10]

Homozygous tottering mice also have abnormal electrocorticograms (ECG) with 6/sec spike and waves occurring hundreds of times daily [7,12] The ECG changes are accompanied behaviorally by a brief cessation in activity and do not appear temporally linked to the more pronounced GDSC Disturbances of swimming ability, rotorod performance, and open field activity are also associated with phenotypic expression of the *tg* mutation [22] GDSC were not induced by auditory, photic, somatosensory, or vestibular stimulation in C57BL/6J*(tg/tg)* mice [12] and are thus regarded as "spontaneous "

The pharmacologic response of the GDSC of *(tg/tg)* mice has been studied in this laboratory A procedure is described in which a GDSC can be induced at will in *(tg/tg)* mice Among several anticonvulsant agents tested for activity, only the benzodiazepine diazepam was able to protect *(tg/tg)* mice against PTZ induced GDSC Treatment with phenytoin was found to induce GDSC This is the first report of a treatment that can block the genetically-determined stereotypic convulsions of *(tg/tg)* mice and may provide insight into mechanisms underlying their occurrence

## METHOD

### *Animal Rearing*

Male and female C57BL/6J*(tg/tg)* and *(tg/+)* mice were

obtained from the Jackson Laboratory (Bar Harbor, ME) at the N16 and N17 cross generation and used for breeding Animals were housed in polypropylene cages with pine shaving bedding and free access to food and water at all times Temperature was maintained at 24°C Lighting was on a 12 hr (0800–2000) cycle Brother×sister mating of a homozygous male with a heterozygous female was used to produce *(tg/tg)* and *(tg/+)* offspring Litters were weaned at 4 weeks of age, housed according to sex, and observed periodically to identify homozygotes A homozygous mouse was permanently marked by ear punch when the 'tottering" gait or stereotypic convulsions were first observed but remained housed with littermates Over 100 *(tg/tg)* mice have now been studied in this fashion with only one instance of an animal not being identified as homozygous prior to 10 weeks of age Therefore, it appears unlikely that a *(tg/tg)* was falsely identified as a *(tg/+)* animal

The subjects used in these studies were 18 *(tg/tg)* 14 *(tg/+)*, and 5 wild type C57BL/6J(+/+) mice of both sexes The ages of individual litters used in an experiment are given in the legends of the corresponding table In general, mature adults with well defined seizures were used Whenever possible comparisons were made between littermates of the same sex

### *Seizure Threshold Determination*

A tail vein infusion method [6] was used to measure the threshold dose for PTZ-elicited convulsions in unrestrained mice The barrel from a 27 gauge needle was removed and fitted with a length of PE 50 tubing connected to a 1 cc tuberculin syringe mounted on a hand operated micro-drive The system was filled with a 0 3% solution of PTZ in 0 9% NaCl and slowly infused into a tail vein until the animal responded The volume infused was measured and the animal's weight determined so that a dose, in mg/kg, could be calculated Mice were confined to a one liter glass beaker during the infusion and subsequent convulsive period The response measured was either a clonic convulsion with the loss of righting or the start of a GDSC, whichever occurred first

### *Induction of GDSC by PTZ*

The following procedure was developed to reliably induce a GDSC in *(tg/tg)* mice Animals were brought to the laboratory on the morning of the experiment in their home cages, weighed, and then left undisturbed for at least 60 min to adjust to the laboratory setting "Spontaneous" GDSC were seen by casual observation 21 of 96 possible times during this period After the adjustment period mice were pretreated with the test agent or vehicle (see below) and returned to their cage Animals were observed 10 min later and at regular intervals thereafter for signs of drug action and the occurrence of GDSC At designated times (see below), corresponding for each agent to a time of known anticonvulsant action as reported in the literature, animals were injected intraperitoneally with 30 mg/kg of PTZ in 0 9% NaCl (10 ml/kg body weight) and placed in individual observation chambers (30×9×10 cm, L×W×H) for 60 min In addition to noting the presence or absence of a GDSC the following data were usually recorded (1) time of onset of first sign of PTZ action, (2) time of onset of "partial" and/or complete GDSC (see Results section for description) and (3) duration of complete GDSC, up to 60 min The first sign of PTZ action was taken as the first noticeable change in behavior following administration of the PTZ It could be either twitching, a startle response, "partial" GDSC, or complete GDSC Littermate *(tg/+)* mice were treated using identical procedures and observed along with homozygotes Heterozygotes served as controls for strain-specific, gene dosage or idiocyncratic drug responses Convulsions were induced between 1200 hr and 1500 hr except when aminooxyacetic acid (AOAA) was tested 6 hr post-treatment

### *Drug Treatments*

All drugs and their vehicles were administered by the intraperitoneal route in a volume of 5 ml/kg body weight The vehicle for ethosuximide, AOAA, and naloxone was 0 9% NaCl For phenytoin, sodium phenobarbital and sodium valproate it was 0 9% NaCl, pH 11 and for diazepam and valproic acid it was propylene glycol ethanol water (40 10 50 by volume) The intervals between drug treatment and induction with PTZ were as follows 0 9% NaCl, 0 5 h, ethosuximide, 0 5 h, valproic acid, 0 5 hr, sodium valproate, 0 5 hr, naloxone, 1 hr, 0 9% NaCl, pH 11, 2 hr, sodium phenobarbital, 2 hr, phenytoin, 2 hr, diazepam vehicle, 2 hr diazepam, 2 hr, AOAA, 1 5 or 6 hr

Pentylenetetrazol AOAA, and propylene glycol were purchased from the Sigma Chemical Co (St Louis, MO) The following drugs were gifts from the manufacturers naloxone HCl (Endo Laboratories, Inc ), diazepam (Hoffmann-La Roche, Inc ), phenytoin and ethosuximide (Park, Davis and Co ), sodium phenobarbital (Sterling Drug, Inc ), valproic acid (Abbott Laboratories) All doses refer to drugs in the form specified above except sodium valproate which is given as the free acid Valproic acid was converted to the sodium salt by the addition of an equal volume of equimolar NaOH

Four groups of mice were used in testing agents for anticonvulsant activity Because of a shortage of homozygous mice animals were retested with different agents At least 7 days, and usually 10–14 days or longer, elapsed between testing animals with a new agent One group of 4 *(tg/tg)* mice received diazepam vehicle, diazepam, phenytoin vehicle phenytoin (25 mg/kg), naloxone vehicle, naloxone, ethosuximide, phenobarbital, sodium valproate and AOAA (6 hr) A second group of 4 *(tg/tg)* mice received naloxone vehicle, naloxone, diazepam vehicle, diazepam, phenytoin vehicle, phenytoin, ethosuximide, phenobarbital, and AOAA (1 5 hr) A third group of 3 *(tg/tg)* mice received AOAA (6 hr), valproic acid, sodium valproate, phenytoin (50 mg/kg) and phenobarbital (50 mg/kg) The last group, which contained one *(tg/tg)* mouse, was tested with AOAA (6 hr) only

Blood levels for the drugs used were not measured in these experiments since it was necessary to re-use the subjects on several occasions Data from other sources [5] as well as indirect data (see Results section) indicate that effective anticonvulsant blood levels result from the doses and times used in these studies

## RESULTS

### *Induction of GDSC with PTZ*

Complete stereotypic behavioral seizures (i e , a GDSC) were reported observed within 3–5 min following the injection of 20 mg/kg PTZ into *(tg/tg)* mice [12], however, this finding was not reproducible in this laboratory (data not pre-

TABLE 1

THRESHOLD DOSES FOR CONVULSIVE RESPONSE TO PENTYLENETETRAZOL IN C57BL/6J TOTTERING MOUSE GENOTYPES

| Response elicited | Mean ± S D * | Genotype |
|---|---|---|
| GDSC | 29 5 ± 14 6† | (*tg/tg*) (6) |
| Generalized clonic convulsion | 51 5 ± 12 7 | (*tg/+*) (8) |
| Generalized clonic convulsion | 51 8 ± 7 3 | (+/+) (5) |

*Units are mg/kg

†F(2,16)=6 684, $p<0$ 01, one-way analysis of variance, $p<0$ 01 (*tg/tg*) vs (+/+), 2-tail *t*-test, $p<0$ 02 (*tg/tg*) vs (*tg/+*), 2-tail *t*-test

Animals from 7 litters were used Nine mice were male (5 *tg/tg*, 4 *tg/+*) and 5 were female (1 *tg/tg*, 4 *tg/+*) Wild type (+/+) were all males from 2 litters Tottering litters were 117, 119, 125, 134, 135, 150, or 154 days old when tested Wild type mice were 76 or 98 days old No differences due to sex or age were apparent Number of animals tested in parentheses

sented) In order to determine whether any dose of PTZ was able to induce GDSC in (*tg/tg*) mice from our breeding colony, the threshold dose needed to elicit any convulsive response in tottering mice by PTZ was examined (Table 1) Our results are in qualitative agreement with [12] in that GDSC were induced in (*tg/tg*) mice by PTZ at a mean dose (29 5 mg/kg) significantly lower ($p<0$ 01) than the doses that elicit generalized clonic convulsions in (*tg/+*) and (+/+) mice (Table 1)

*Responses of Tottering Mice to 30 mg/kg PTZ*

Based upon this data (Table 1) the injection of 30 mg/kg PTZ IP was found to reliably induce GDSC in (*tg/tg*) mice Using this procedure 23 of 24 (*tg/tg*) mice have been successfully induced following a first injection of 30 mg/kg PTZ The lone non-responder was not induced even after 6 separate injections Two types of responses have been observed following administration of 30 mg/kg PTZ to (*tg/tg*) mice The most common response is a complete GDSC lasting 40–50 min which is indistinguishable from a "spontaneous" GDSC The second response is what appears to be a "partial" convulsion In this case the animal begins to show the stereotypic movements associated with initiation of a GDSC (flattening of the sacrum, hindlimb abduction and dorsiflexion, and flexion of the head) but these are aborted within a few seconds Several attempts at a GDSC usually occur over a 5 min period Normal activity or, less commonly, PTZ induced twitching is seen between "partial" GDSCs

A third response has occasionally been observed where an animal has only twitches, contractions, and a marked Straub tail in response to the PTZ These effects can be attributed to PTZ acting at a site other than the mutation-induced site, since they have also been seen in other strains of mice after repeated injections with low doses of PTZ [23] A fourth response not seen in (*tg/+*) and DBA/2J mice is hypo-activity following 30 mg/kg PTZ The animal lies down and remains inactive for 10–20 min

*Protection Against PTZ Induced GDSC*

Several antiepileptic drugs have been tested for their abilities to block GDSC induced by 30 mg/kg PTZ using the procedure described in the Method section (Table 2) Ethosuximide, valproic acid, phenobarbital, and phenytoin were unable to block the induction of GDSC by PTZ Surprisingly, phenytoin reliably induced GDSC in (*tg/tg*) mice (Table 2) This effect was evident within 10–15 min of administration of the phenytoin A similar, but less reliable effect was also seen with 250 mg/kg ethosuximide Neither the opiate antagonist naloxone nor the GABA-elevating drug AOAA had any effect on induction of GDSC by PTZ

Although (*tg/tg*) mice were not protected against GDSC at the doses used, evidence of pharmacologic action was generally observed Sedation and mild to severe ataxia following phenytoin, phenobarbital, valproic acid, and sodium valproate were observed at the time of testing For ethosuximide, the presence of pharmacologic activity was demonstrated by its ability to block generalized clonic-tonic convulsions in these mice (see below)

The only agent tested that blocked PTZ induced GDSC in (*tg/tg*) mice was the benzodiazepine diazepam Two hours after a 4 mg/kg dose 100% of the mice tested were protected The animals were awake and moving at this time but showed intensified ataxia that was not abolished by PTZ treatment In contrast, heterozygotes were also ataxic but this action of diazepam was antagonized by 30 mg/kg PTZ

*Effect of Drug Treatment on Parameters of PTZ Induced GDSC*

Table 3 shows values for the following components of PTZ induced GDSC in (*tg/tg*) mice (1) time of onset of first sign of PTZ action, (2) time of onset of complete GDSC, (3) time of onset of "partial' and/or complete GDSC and (4) duration of complete GDSC Not all animals had every response The duration of convulsion is presented only for those mice which finished within the 60 min observation period Any animal still experiencing a GDSC at the end of the period was assigned a lower limit for duration but was not included in the calculations Incomplete records were also kept for some of the first drugs tested so that no data is available

It can be seen (Table 3) that the various treatments generally had little effect on these parameters The only significant differences between treatments were in the time of onset of complete GDSC A delay of onset was recorded following treatment with naloxone or 0 9% NaCl, pH 11 The apparent effect of the pH 11 vehicle on latency seemed to be anomolous since it is not reproducible (data not shown)

*Protection Against Generalized PTZ Convulsions*

Since several of the agents ineffective against the GDSC in (*tg/tg*) mice are effective in blocking generalized clonic-tonic convulsions produced by a larger dose of PTZ [8], the following experiment was performed to determine whether homozygous mice can be protected against generalized PTZ convulsions

Two groups of eight mice (4 *tg/tg*, 4 *tg/+*) were treated with 0 9% NaCl or 250 mg/kg ethosuximide in saline 30 min prior to challenge with a large dose of PTZ (85 mg/kg, SC) When saline pretreated homozygotes received PTZ they responded with a mixture of GDSC (dorsiflexion of hindlimbs, flexion of the neck, and clonus in fore- or hindlimbs) and generalized PTZ convulsions (intense tremor, marked Straub

TABLE 2

EFFECT OF DRUGS ON PTZ INDUCED GDSC IN (*tg/tg*) MICE

| Treatment | Number showing spontaneous GDSC* | Number showing partial GDSC | Number showing complete GDSC | % Protected† |
|---|---|---|---|---|
| 0 9% NaCl (5 ml/kg) | 1/8 | 2/8 | 6/8 | 0% |
| Ethosuximide (250 mg/kg) | 5/8‡ | 0/8 | 8/8 | 0% |
| Aminooxyacetic acid (25 mg/kg 6 hr) | 0/8 | 0/8 | 6/8 | 25% |
| Aminooxyacetic acid (25 mg/kg 1 5 hr) | 2/4 | 0/4 | 4/4 | 0% |
| Naloxone (6 mg/kg) | 0/8 | 1/8 | 7/8 | 0% |
| 0 9% NaCl pH 11 (5 ml/kg) | 0 8 | 1/8 | 7 8 | 0% |
| Phenytoin (25 mg/kg) | 8/8§ | 3/8 | 4/8 | 12 5% |
| Phenytoin (50 mg/kg) | 3/3¶ | 3/3 | 0/3 | 0% |
| Phenobarbital (25 mg/kg) | 1/8 | 0/8 | 8/8 | 0% |
| Phenobarbital (50 mg/kg) | 0 3 | 0/3 | 3/3 | 0% |
| Sodium valproate (200 mg/kg) | 0/7 | 1 7 | 6/7 | 0% |
| Propylene glycol ethanol water (5 ml/kg) | 0/8 | 1/8 | 5/8 | 25% |
| Diazepam (4 mg/kg) | 0/8 | 0/8 | 0/8 | 100%# |
| Valproic acid (150 mg/kg) | 0/4 | 0/4 | 3/4 | 25% |

* 'Spontaneous GDSC refers to a GDSC occurring after drug treatment and prior to induction with PTZ

†Calculated as percent of animals failing to show a 'partial or complete GDSC

‡$p<0$ 04 versus 0 9% NaCl 2-tail test of differences between sample proportions [3]

§¶$p<0$ 01 versus 0 9% NaCl, pH 11, 2-tail test of difference between sample proportions [3]

#$p<0$ 01 versus propylene glycol ethanol water 2-tail test of difference between sample proportions [3]

Mice from 5 different litters were studied Ten mice were female (7 *tg/tg* 3 *tg/+*) and 8 male (5 *tg/tg* 3 *tg/+*) Litters were 139 144, 290 299, or 302 days of age when testing began Testing ended 111 or 112 days later, except for the youngest litter which ended 9 days later Only data for the homozygotes are presented (see Method section for further details)

tail, generalized clonus and hopping) The ethosuximide-treated homozygotes, on the other hand, all went into a complete GDSC with only slight signs of generalized PTZ action (an occasional weak Straub tail with localized brief twitching) All saline treated (*tg/+*) mice had generalized PTZ convulsions and 2 of 4 mice died during tonic hindlimb extension The ethosuximide treated (*tg/+*) mice were all protected with only an occasional twitch observed These mice had strabismus and moved very little

## DISCUSSION

The genetic mutation designated tottering confers upon the recipient homozygous mouse an altered nervous system which periodically undergoes aberrant activity resulting in "spontaneous" stereotypic behavioral convulsions (GDSC) These genetically-determined stereotypic convulsions may also be elicited by exposure to a dose of PTZ which is subconvulsant in heterozygotes and normal mice of the same strain (Table 1)

The site of action for this effect of PTZ is unknown but appears to be distinct from the site(s) whereby PTZ induces generalized clonic-tonic convulsions in mice This conclusion is based upon the observation that ethosuximide, phenobarbital, valproic acid, and AOAA, agents that block convulsions by a higher dose of PTZ [8 16] were ineffective in altering the action of 30 mg/kg PTZ on (*tg/tg*) mice This conclusion was strengthened by a test for generalized anticonvulsant action using ethosuximide (see Results section) It is possible that altered pharmacodynamics are responsible for the results since only one or two doses of each drug were tested, however, the doses and time of testing were chosen to be within the range for good anticonvulsant activity in mice for each drug used [8, 13 16] Therefore, the former interpretation appears more likely

If we may generalize from the rat two mechanisms for PTZ's induction of GDSC may be proposed One possibility is that 30 mg/kg PTZ acts as a discriminative stimulus (DS) that mimics the natural interoceptive stimuli normally eliciting the GDSC response Using a classical lever-pressing paradigm it has been shown that 20 mg/kg PTZ in the rat is a DS [17] This property of PTZ does not generalize to most other convulsants and is not antagonized by ethosuximide or phenytoin among other anticonvulsants [19] Only those anticonvulsants which also have anxiolytic activity were able to antagonize the DS property of PTZ [19,20] It has been proposed that the DS property of PTZ results from an anxiogenic action [18] Data from this laboratory suggest that "spontaneous" GDSC may be induced by stress or fear (see below) In this respect the ability of diazepam to antagonize PTZ induced GDSC in (*tg/tg*) mice (Table 2) would not be surprising

A second possible mechanism involves the ability of subconvulsant doses of PTZ as well as other convulsants, to enhance sensory evoked potentials in the mesencephalic reticular system [1] Following an injection of 30 mg/kg PTZ sensory pathways that elicit the "spontaneous" GDSC may be enhanced such that one occurs in the presence of the PTZ i e PTZ is permissive to the expression of a 'spontaneous ' GDSC

TABLE 3

EFFECT OF TREATMENT ON PARAMETERS OF PTZ INDUCED GDSC

| Treatment | Time of onset of first sign of PTZ action | Time of onset of complete GDSC* | Time of onset of partial and/or complete GDSC | Duration of complete GDSC |
|---|---|---|---|---|
| 0 9% NaCl | 2 26 ± 0 89 (8) | 4 75 ± 1 17 (6) | 4 17 ± 0 45 (8) | 51 15 ± 9 43 (5) |
| Ethosuximide | 3 34 ± 1 10 (4) | 4 96 ± 2 84 (4) | 3 34 ± 1 10 (4) | 41 0 |
| Aminooxyacetic acid (6 hr) | 5 69 ± 7 90 (7) | 6 96 ± 7 87 (6) | 5 38 ± 7 79 (6) | 35 30 ± 13 57 (5) |
| Aminooxyacetic acid (1 5 hr) | 1 75 ± 0 61 (4) | 4 21 ± 9 30 (4) | 1 96 ± 0 41 (4) | 27 88 ± 7 05 (4) |
| Naloxone | 3 53 ± 3 61 (8) | 14 28 ± 7 91 (7) | 3 60 ± 3 28 (8) | 37 38 ± 4 42 (2) |
| 0 9% NaCl, pH 11 | 2 67 ± 2 00 (8) | 17 62 ± 13 30 (8) | 10 64 ± 12 57 (8) | 42 89 ± 8 57 (3) |
| Phenytoin (25 mg/kg) | 2 12 ± 0 76 (8) | (no data) | 2 25 ± 0 71 (7) | (no data) |
| Phenytoin (50 mg/kg) | 2 77 ± 0 25 (3) | (no data) | 12 18 ± 2 04 (3) | none |
| Phenobarbital (25 mg/kg) | 4 04 ± 3 19 (8) | 5 98 ± 3 04 (7) | 4 31 ± 3 30 (7) | 44 87 ± 7 58 (8) |
| Phenobarbital (50 mg/kg) | 6 64 ± 2 33 (3) | 9 58 ± 4 98 (3) | 6 64 ± 2 33 (3) | 46 67 |
| Sodium valproate | 2 55 ± 1 03 (7) | 2 95 ± 0 49 (6) | 7 71 ± 14 6 (7) | 38 21 ± 7 76 (6) |
| Propylene glycol ethanol water | 3 55 ± 1 29 (4) | 6 61 ± 0 28 (4) | 4 49 ± 0 91 (4) | (no data) |
| Diazepam | none | none | none | none |
| Valproic acid | 2 73 ± 0 79 (3) | 5 14 ± 0 66 (2) | 4 55 ± 1 48 (2) | 56 20 |

*One way analysis of variance showed a significant difference between treatments ($p<0$ 001)

Values are in min (mean ± SD) with sample size shown in parentheses Data were collected from the same group of animals used in Table 2 "none means no animals showed that particular response following treatment Analysis of variance did not include treatment groups with "none ' values

At the present time, no sensory modality has been identified that can elicit GDSC in *(tg/tg)* mice Unpublished studies from this laboratory may provide some clues as to an adequate stimuli for the appearance of "spontaneous" GDSC It has been found that ' spontaneous" GDSC occur approximately 50% of the time when *(tg/tg)* mice are repeatedly watched continuously for 60 min by a human observer On the other hand, the occurrence of "spontaneous" GDSC was much lower (<5% of the time) in animals observed repeatedly using video camera recordings (Syapin and Segal, unpublished data) These data suggest that anxiety, stress, or fear triggered by the presence of the observer may induce a "spontaneous" GDSC

The spike and wave ECG activity in *(tg/tg)* mice, with the concurrent brief behavioral immobility, has led some authors to propose that *(tg/tg)* mice have two independent ictal events, motor convulsions (GDSC) and ' absence" seizures [7] On the other hand, the two events may stem from a common mechanism The genetic mutation tottering arose in the DBA/2J strain of mouse [4], a strain that is also known to have spontaneous, bilaterally hypersynchronous spike bursts in their ECG [15] These ECG abnormalities are also induced by subconvulsant doses of PTZ and are accompanied by active suppression of body movement [15] It seems plausible that the *tg*-mutation could lead to a threshold reduction that allows these ECG changes to occur spontaneously "

A means to clarify the relationship between the spike and wave activity and the GDSC is to investigate the effect of drugs on their respective activities If these are independent ictal events, it should be possible to suppress one without affecting the other If one event is an epiphenomenon of the other, blocking one should suppress the other In a preliminary report [5] phenytoin at 30 or 60 mg/kg was ineffective in altering the spike and wave activity *(tg/tg)* mice Valproic acid (100 mg/kg) was found to reduce spike and wave activity for up to 40 min after intraperitoneal injection, while repeated injections every 30 min for 6 hr led to complete absence of spike and wave activity for 6 hr How these results relate to the present findings is not clear since stereotypic convulsions were not measured or induced Further study with direct monitoring of both events is necessary to adequately answer this question

The present results may provide some clues to the neurochemical mechanism of GDSC expression in *(tg/tg)* mice The lack of protection by naloxone (6 mg/kg) suggests that endogenous opioids do not participate in the generation of the GDSC response Likewise, the failure of 25 mg/kg AOAA to alter convulsions after either 6 hr, the time of peak elevation of brain synaptosomal GABA content [24], or 1 5 hr, a time of maximal anticonvulsant activity [9], suggests that GDSC are not due to a reduced level of GABA or influenced by increased GABA levels The lack of effect with both sodium valproate and valproic acid is consistant with this conclusion However, further testing with different doses or continuous administration of these drugs is needed before final conclusions can be drawn

The results with diazepam may indicate an involvement of the high affinity benzodiazepine receptor system [21] in these convulsions The dose and time after administration of diazepam used in the present study was the same as previously shown to occupy saturable, high affinity brain benzodiazepine receptor sites *in vivo* and to protect completely against generalized clonic-tonic convulsions induced by 80 mg/kg PTZ [13] *In vitro* evidence from rats [11] and mice [23] has demonstrated a competitive inhibition between PTZ and diazepam at the high affinity receptor site Therefore, one might conclude that diazepam blocked GDSC in *(tg/tg)* mice by inhibiting PTZ from interacting with a benzodiazepine receptor site Studies in this laboratory also indicate

that "spontaneous" GDSC can be stopped by treatment with diazepam (unpublished observation)

The results with phenytoin (Table 2) are interesting since it has been proposed [2] that the ability of phenytoin to interact with benzodiazepine receptors is responsible for some of the anticonvulsant action of this drug However the results of this study suggest that this relationship is altered due to the tottering mutation In *(tg/tg)* mice, phenytoin appears to act similarly to PTZ and was found to induce GDSC (Table 2)

The present study demonstrates that *(tg/tg)* mice would be useful as models for human epilepsy These animals have a recessive genetic trait which predisposes them to a life of convulsions that occur as a result of normal, everyday activity GDSC in *(tg/tg)* mice have several features in common with human simple partial seizures [14] (1) the abnormality seems localized to distinct neuronal pools, hence stereotypy, (2) the seizures are "inappropriate responses", (3) the animal remains conscious throughout the convulsion, and (4) benzodiazepines are an effective treatment for GDSC and some types of human partial seizures Besides the concurrent presence of an ataxic gait, the mutation seems to do little else to hinder the mouse unless the neurological competence of the animal is challenged [22] As a means to study at least one genetic component of seizure susceptibility the tottering mutation appears well suited and deserved further attention

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