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Thiosemicarbazide Induces Partial Myoclonic, Generalized Tonic-Clonic and Status Epilepticus Seizures Depending on the Dose in the Rat

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Abstract: The present study aimed to characterize the seizures produced by injections of Thiosemicarbazide (TSC), a glutamic acid decarboxylase inhibitor. It aimed also to evaluate the anticonvulsant effect of the antiseizure medications Phenobarbital (PHB), Valproate (VPA), and Phenytoin (PNT) and of the antipsychotics Chlorpromazine (CPZ) and Haloperidol (HAL) as well as the antidepressant SSRI, Fluoxetine (FXT). Male Wistar rats received TSC i.p. injections of 2.5, 5, 7.5, 10, and 20 mg/kg. The rat's changes in behavior and the onset latencies of seizures were recorded and compared using an ANOVA test whereas the incidences of seizures were compared using a χ^2 test. Seizure severity was categorized as Partial Myoclonic Seizures (PMS), Generalized Tonic-Clonic Seizures (GTCS), and Status Epilepticus (SE). At low doses, TSC induced a significant change in behavior indicating an anxiety-inducing effect. The threshold dose for PMS was 7.5 mg/kg and when the dose increased to 10 mg/kg, PMS progressed to GTCS. TSC 7.5 mg/kg dose also induced susceptibility to audiogenic seizure ($p<0.005$) while 10 and 20 mg/kg doses caused SE in a dose-dependent manner ($p<0.05$). PHB (30 mg/kg), VPA (200 mg/kg), PNT (30 mg/kg), and VPA + PNT (100+15 mg/kg) prevented SE and significantly reduced the incidence of TSC 10 mg/kg-induced PMS ($p<0.05$) and GTCS ($p<0.001$). These treatments also increased the onset latencies of seizures ($p<0.001$). HAL (1 mg/kg), CPZ (1 and 2 mg/kg), and FXT (1 and 2 mg/kg) significantly reduced the incidence of PMS ($p<0.05$) and GTCS ($p<0.01$). The occurrence of SE was completely inhibited by FXT ($p<0.001$). All doses of HAL, CPZ, and FXT increased onset latencies of GTCS ($p<0.01$) while HAL (1.5 mg/kg), CPZ (2 mg/kg), and FXT (1 and 2 mg/kg) also increased onset latencies of PMS ($p<0.05$). Our study validated TSC-induced seizures in rats as a dose-dependent model of PMS, GTCS, and SE.

Keywords: Thiosemicarbazide, Seizures, Rat, Antiseizure Drugs, Psychopharmacologic Drugs

Introduction

Animal models of seizures used in preclinical experiments provide the "basis" for the development of new Antiseizure Medications (ASMs), previously referred to as antiepileptic drugs. Knowledge of the actions and characteristics of ASMs was generated from experiments using acute animal models such as Pentylenetetrazole (PTZ) and Electroshock (MES) (Löscher and Shmidt, 1994) and chronic models such as kainic acid (Ben-Ari *et al*., 1980), pilocarpine (Leite *et al*., 1990) and kindling (McNamara, 1995). Furthermore, the study of the specific efficacy against various seizures, the characterization of the spectrum of anticonvulsant activity, and the changes in the effectiveness during chronic treatment also required the use of these models. However, the progress made in preclinical experiments has not yet brought solutions to certain clinical situations.

Indeed, 30% of patients are still pharmacoresistant (Chen *et al*., 2018) and many first and second-generation, as well as new ASMs, induce significant adverse effects in patients that limit their clinical use (Kwan and Brodie, 2000; Hanaya and Arita, 2016).

Models of epilepsy and epileptic seizures have been well characterized and some of them, namely MES and PTZ, can predict antiepileptic efficacy in humans (Yuen and Trocóniz, 2015). However, although the efficacy of pharmacotherapy has improved with the discovery of new ASMs, the MES and PTZ models are not always able to reveal the effects of potential ASMs. Thus, some compounds that are potentially effective but act through mechanisms not yet explored cannot be revealed (Barker-Haliski, 2019). For this reason, the characterization of other models of epilepsy and seizures would still be useful in the development of new ASMs. In some models of chemically induced seizures, systemically administered drugs act as GABAA antagonists. Likewise, Glutamic Acid Decarboxylase (GAD) inhibitors, which inhibit GABA synthesis, are also potent chemo-convulsants (Horton and Meldrum, 1973; Velišek, 2009). GABAA antagonists have been well studied, while none of the GAD inhibitors have been commonly used in experimental epileptology or in pharmacological screening. Thiosemicarbazide (TSC) is a drug that inhibits GAD (Collins, 1973), lowers GABA levels in the central nervous system (Roa *et al*., 1964; Sze *et al*., 1971), and causes seizure discharges in cats (Dunlop *et al*., 1960), cerebral excitation in dogs (DanteRoa *et al*., 1964) and convulsions in mice (Golovenko *et al*., 2017). The effect of TSC, however, has not been characterized behaviorally despite these existing data. In a previous study, the effects of TSC were evaluated following intracerebral microinjections in rats and the results showed that the elicited behavioral responses were similar to those caused by GABAA receptors antagonists when microinjected into the same cerebral sites (Bagri *et al*., 2007). We, therefore, conducted the present study to characterize the seizures that could be caused by intraperitoneal injections of TSC in rats. The use of the rat was justified by the fact that the rat is considered an important model in the development of preclinical drugs and that active molecules that can become drugs are regularly tested in rats to assess their safety and efficacy before clinical trials in humans (Gill III *et al*., 1989).

In the process of validating an animal model, it is necessary to ensure that the pharmacological reactivity is similar to that observed with effective treatment in human clinics. First-generation ASMs such as Phenobarbital (PHB) and Phenytoin (PNT) as well as second-generation Valproate (VPA) have been shown to be effective clinically and against various seizure models (Löscher, 2011). However, there are some differences in their effects that could be due to differences in the mechanisms underlying the development of epileptogenesis (Sasa, 2006). There is also variation in active doses between different ASMs depending on the seizure model which shows the importance of testing the same compound for its anticonvulsant efficacy in different tests. Therefore, in the second part of our study, we evaluated the anticonvulsant effect of the ASMs, PHB, VPA, and PNT on seizures induced by TSC.

In clinical practice, Psychopharmacological Drugs (PPDs) have been associated with a risk of the onset of seizures. Antipsychotics (APDs) are known to reduce the seizure threshold and cause seizures. Chlorpromazine (CPZ) has been associated with an increased risk of causing seizures while Haloperidol (HAL) has been associated with a lower risk (Hedges *et al*., 2003; Pisani *et al*., 2002). SSRIs antidepressants have also presented a risk factor for seizures with an incidence of 0.1 to 4% at therapeutic doses (Torta and Monaco, 2002) but some data from controlled clinical studies with Fluoxetine (FXT) have suggested a possible anticonvulsant action (Favale *et al*., 1995). In animal models, some differences exist when these drugs have been tested for their ability to affect seizures (Kretzschmar and Teschendorf, 1980). Therefore, in the third part of our study, we also extended the pharmacological characterization of the TSC model to the evaluation of the effects of two APDs, CPZ and HAL, and of an SSRI antidepressant, FXT, on TSC-induced seizures.

Materials and Methods

Animals and Drugs

Male Wistar rats weighing about 200 g at the start of the experiment were used. They were housed in 3 per cage with access to water and food ad libitum. The environmental conditions in the laboratory animal facility were a 12/12h light/dark cycle and a temperature of 21°C. Rats were treated in compliance with the guidelines of the Hassan 1th university. All procedures were in accordance with the European decree, related to the ethical evaluation and authorization of projects using animals for experimental procedures, 1 February 2013, nor: AGRG1238767A. TSC was purchased from Fluka while ASMs [Phenobarbital (PHB), sodium Valproate Acid (VPA), and 5-diphenylhydantoin (PNT)] were purchased from Sigma. Doses of drugs were prepared 30 min before each experiment. The drugs were dissolved in distilled water and a volume of 1-2 mL was injected intraperitonealy into the tested rats.

Selection of Rats Non-Susceptible to Audiogenic Seizure

Rats used in all experiments were tested for susceptibility to Audiogenic Seizure (AGS) according to Bagri *et al*. (1989a) with some modifications. Briefly, each rat was exposed to acoustic stimulation for 90 sec in an

acoustically isolated box (45 × 45 × 45 cm) equipped with a transparent front door. The sound was delivered via a sound amplifier connected to two loudspeakers placed on the ceiling of the box. The software was used to select the parameters of the acoustic stimulation (frequencies 20, 30, 40 Khz, and 120-160 dB of intensity). Each rat underwent two separate 48 h test sessions and changes in behavioral responses were noted with emphasis on the possible onset of Wild Running (WR) (number of episodes and latency), convulsive seizures (back flexion, tonic extension of the limbs, and myoclonic jerks) and a phase of quiescence.

Assessment of TSC-Induced Seizures: Intensity and Onset Latency

Six groups of 6 rats each were used for this experiment. Animals in each group received an i.p. injection with one of the following doses of TSC: 0, 2.5, 5, 7.5, 10 and 20 mg/kg. After each injection, the rat was placed in an open-field arena. The rat's behavior was observed by the experimenter for a 30 min period. The quantification concerned the elements of natural behavior but also the different behavioral categories of seizures. Seizure onset latencies were recorded with a cutoff latency of 240 min.

The assessment of the intensity of seizures was performed using a categorization of seizure behaviors. Preliminary observations showed that the clinical features of the TSC seizure model could not be adequately described based on the widely used Racine seizure scale for the amygdala-stimulated model in the rat (Racine, 1972). This is because certain stages of behavioral intensity mentioned in the Racine scale such as "head nodding", "rearing" and "rearing associated with a fall" were not observed. Comparing our observations to other seizure models, we noted that the seizures induced by TSC were closely similar to what has been described for seizures induced by GABAA antagonists, including the PTZ model. To characterize the intensity of the seizures, we, therefore, used the behavioral score for seizures induced by PTZ developed by Lüttjohann *et al*. (2009). This scale also made it possible, based on the EEG recording, to distinguish partial seizures from secondarily generalized seizures. We categorized the severity of seizures observed after TSC treatment as follows: Partial Myoclonic Seizures (PMS) corresponding to stages 1, 2, 3, and 4 of the (Lüttjohann *et al*., 2009) scale and Generalized Tonic-Clonic Seizures (GTCS) corresponding to stages 5 and 6 of the same scoring scale. We also considered the occurrence of Status Epilepticus (SE) when a GTCS persisted and was recurrent for more than 8 min according to the description reported by the international league against epilepsy.

Elicitation of AGS Susceptibility

Thirty minutes after an i.p. injection of TSC, each rat was subjected to an AGS sensitivity test according to the procedure described in paragraph 2.2. The experimenter noted the possible appearance of WR and convulsions as well as their latencies.

Pretreatment with ASMs

To test the effects of ASMs on TSC-induced seizures, rats were divided into 5 groups of 6 rats. We selected ASMs doses that have proved their efficiency in other rat models of seizures (Bagri *et al*., 1991), and a single 1 to 2 mL ASMs solution was injected i.p. as a pretreatment to each rat of 4 groups every day for 5 consecutive days in order to maintain effective drug level (Löscher, 2007). Rats of the fifth group were injected with Distilled Water (DW) in the same way as rats of the other groups. Group 1 received PHB (30 mg/kg), group 2 received VPA (200 mg/kg), group 3 received PNT (30 mg/kg) whereas group 4 received a combined dose of VPA and PNT [VPA (100 mg/kg) + PNT (15 mg/kg)]. On the fifth day, a dose of 10 mg/kg of TSC was also injected i.p. 15 min following each ASM or control pretreatment. The seizure protection provided by each ASM was evaluated based on its ability to inhibit PMS, GTCS, and SE.

Pretreatment with PPDs

To test the effects of PPDs on TSC-induced seizures, rats were divided into 7 groups of 6 rats before they received pretreatment with the same protocol as for ASMs. A single dose/day of HAL, CPZ, or FXT was administered to each rat of the six groups for 5 days. Groups 1-2 received pretreatment with HAL at doses 1 and 1.5 mg/kg respectively. Groups 3-4 received CPZ at doses 1 and 2 mg/kg whereas groups 5-6 received FXT at doses 1 and 2 mg/kg respectively. Group 7 received pretreatment with control injection (DW). On the fifth day, a dose of 10 mg/kg of TSC was also administered 15 min following each PPD pretreatment. The seizure protection provided by PPDs was evaluated based on their ability to inhibit PMS, GTSC, and SE.

Statistical Analysis

Behavioral categories were expressed as mean ± SEM and comparisons were made using the ANOVA test. The seizure incidence expressed as a percentage of rats exhibiting PMS, GTCS, or SE- following various doses of TSC, ASMs + TSC, and PPDs + TSC were analyzed using a Chi-square test (χ^2). The onset latencies of seizures were expressed as mean ± SEM and were analyzed by an ANOVA test followed by a Tukey-Kramer post hoc test.

Results

TSC-Induced Changes in Natural Behavior

Administration of TSC induced a significant change in natural behavior (Table 1). With 2.5 mg/kg, tremors of the whiskers and behavioral arrest occurred. The number of rearings was significantly reduced with doses 2.5, 5, 7.5, and 10 mg/kg of TSC ($p<0.01$) while the time spent in scratching was significantly increased with doses 5 and 7.5 mg/kg ($p<0.05$). Time spent in grooming was also increased by 2.5, 5, and 7.5 mg/kg ($p<0.05$). Locomotor activity was significantly reduced after 5, 7.5, and 10 mg/kg ($p<0.01$; $p<0.05$) while exploratory behavior was significantly increased after 2.5, 5, and 7.5 mg/kg ($p<0.01$, $p<0.05$). The estimated urination volume was increased only after 7.5 mg/kg ($p<0.05$).

Elicitation of Clonic and Tonic-Clonic Seizures

Figures 1-2, the threshold dose for the induction of seizures was approximately 7.5 mg/kg. This dose only resulted in the induction of PMS. Behavioral categories of PMS seizures were sudden behavioral arrest and/or motionless staring (2-3 min), salivation and facial twitching expressed with the muzzle or muzzle and eyes (2-3 min), and clonic seizures while the rat is in a sitting position (1 min). When the dose was increased to 10 mg/kg, PMS progressed to GTCS (5 min with interruptions of 15-30 sec). The initial phase of GTCS was WR, which ended in clonic seizures with the rat lying on its side (2 min). Then tonic-clonic seizures appeared with the animal lying on its belly. These were sometimes interrupted by a pure tonic seizure (tonic extension of the limbs). This category of seizures was followed by tonic-clonic seizures while the rat was lying on its side. Finally, wild jumping behavior was observed.

Chi-square analysis with PMS, GTCS, and TSC dose factors (7.5, 10, and 20 mg/kg) showed that there was a significant difference between the three TSC doses with regard to the behavioral seizure categories expressed ($p<0.001$). The occurrence of death began with 10 mg/kg of TSC and was dose-dependent ($p<0.05$), reaching 100% mortality at 20 mg/kg if not treated with diazepam 2 mg/kg.

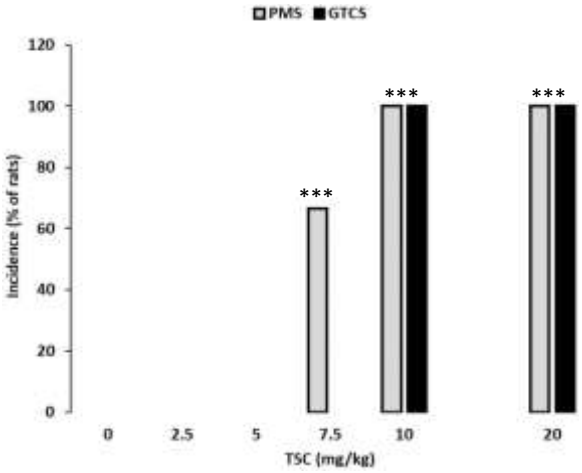

Fig. 1: Incidence (% of rats) of PMS and GTCS induced by doses of Thiosemcarbazide (TSC). Seizures were classified as PMS: Partial Myoclonic Seizure and GTSC: Generalized Tonico-Clonic Seizure. χ^2: ***p<0.001 vs controls

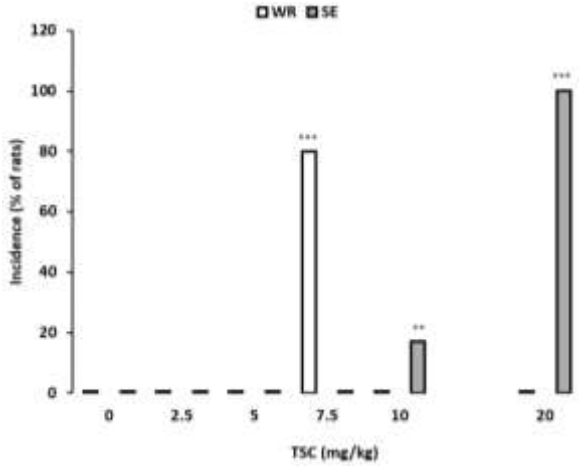

Fig. 2: Incidence (% of rats) of Wild Running (WR) and Status Epilepticus (SE) induced by doses of TSC. χ^2: **p<0.01, ***p<0.001 vs control

Table 1: Effects of thiosemicarbazide intraperitoneal injection on behavior

	TSC (mg/kg)				
Behavioral category	0	2.5	5	7.5	10
Locomotor activity	20.6±2.560	18.6±3.15	11.6±2.22**	15.00±0.79*	15.6±2.62*
Rearing	30.4±3.740	25.7±5.77	13.0±4.33**	21.00±2.98**	10.2±1.59**
Scratching	6.2±2.600	8.1±3.22	11.8±8.9*	17.90±6.7*	7.1±5.10
Urination	26.0±2.520	22.3±2.00	28.0±6.900	89.40±6.30*	24.4±3.99
Grooming	6.6±1.450	10.5±3.1*	11.8±2.12*	18.80±1.23*	7.2±2.32
Exploratory	2.6±0.540	7.5±0.75**	5.8±0.69**	3.60±0.26*	2.2±0.56

Values were expressed as mean ± SEM. ANOVA: *p<0.05, **p<0.01 vs control

Significant difference in the onset latencies of PMS and GTCS were observed between liminar doses (Table 2). A one-way ANOVA with onset latency as dependent variable and seizure category as between-subject factor revealed a significant effect of seizure category ($p<0.01$). Post-hoc pair wise comparisons revealed that the onset latency of GTCS significantly differed from that of PMS ($p<0.01$). Onset latencies of PMS were prolonged after 10 mg/kg only whereas onset latencies of GTCS and SE were prolonged after 10 and 20 mg/kg ($p<0.01$).

Elicitation of AGS Susceptibility and Status Epilepticus

Doses of TSC less than 7.5 mg/kg did not induce apparent sensitivity to AGS in initially non-susceptible rats. However, 7.5 mg/kg induced susceptibility to AGS before the onset of PMS. One or two episodes of WR were triggered during the acoustic stimulation. The χ^2 analysis with WR and TSC-dose factors (2.5, 5, 7 mg/kg) showed that there was a significant difference between the doses of TSC on the elicitation WR ($p<0.001$) (Fig. 2). WR was an explosive run that continued for few seconds. Figure 2 also shows that doses of 10 and 20 mg/kg caused SE in a dose-dependent manner ($p<0.01$ and $p<0.001$ respectively). SE began 8 min after GTCS initiation and lasted for more than 4 h despite an i.p. injection of 2 mg/kg of diazepam, 10 min after the start of SE.

Effect of ASMs on TSC-Induced Seizures

Figure 3 shows the effect of ASMs and the combination of ASMs on the incidence of TSC-induced seizures. PHB (30 mg/kg), VPA (200 mg/kg), PNT (30 mg/kg), and VPA + PNT (100 + 15 mg/kg) reduced the incidence of PMS and GTCS. The χ^2 analysis with the factors PMS, GTCS and PHB, VPA, PNT, and VPA + PNT showed that there was a significant difference between the effects of ASMs on the induced PMS and GTCS ($p<0.001$). The occurrence of SE and mortality was completely inhibited by all ASMs used ($p<0.001$). PHB (30 mg/kg) was the most effective compared to the other drugs ($p<0.05$) and completely prevented the elicitation of TSC-induced PMS and GTCS ($p<0.001$).

PHB, VPA, PNT, and VPA + PNT prolonged onset latencies of PMS and GTCS (Table 3). ANOVA with onset latency as a dependent variable and pharmacological treatment as an inter-subject factor revealed a significant effect of ASMs ($p<0.01$, $p<0.001$). Post-hoc pairwise comparisons revealed that the onset latency of GTCS differed significantly from that of PMS ($p<0.01$).

Effects of PPDs on TSC-Induced Seizures

Figure 4 shows the effect of PPDs on the incidence of TSC-induced seizures. Pretreatment with HAL, CPZ, and FXT resulted in a significant reduction in the incidence of TSC-induced seizures. The χ^2 analysis with the factors PMS, GTCS, and doses (CPZ, FXT) showed that there was a significant difference between the PPDs and the control concerning their effects on the induced PMS and GTCS ($p<0.01$). HAL (1 mg/kg) has a significant reducing effect on PMS ($p<0.01$) and on GTCS ($p<0.05$) while HAL (1.5 mg/kg) has only a significant reducing effect on PMS ($p<0.01$). The occurrence of SE and mortality were completely inhibited by FXT ($p<0.001$).

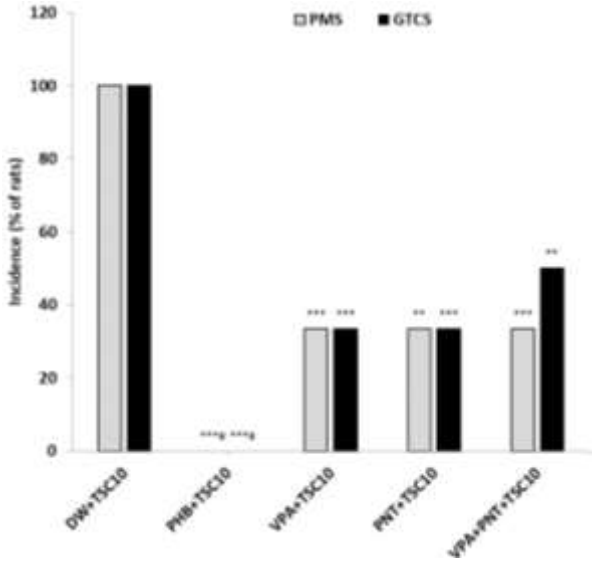

Fig. 3: Effects of pretreatments with ASMs on seizures induced by 10 mg/kg of TSC (TSC10). PMS: Partial Myoclonic Seizure, GTCS: Generalized Tonic-Clonic Seizure, DW: Distilled Water. χ^2: **p<0.01, ***p<0.001 vs control; # p<0.05 vs drugs

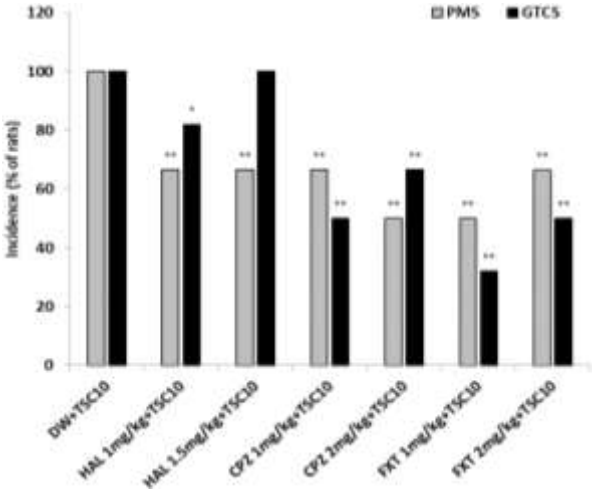

Fig. 4: Effect of pretreatment with psychopharmacologic drugs on TSC-induced seizures. Seizures were classified as PMS: Partial Myoclonic Seizure, GTCS: Generalized Tonico-Clonic Seizure, TSC10: 10 mg/kg of TSC, DW: Distilled Water

Table 2: Variation of seizure onset latencies (mean ± SEM) with the doses of thiosemicarbazide (TSC). PMS: Partial Myoclonic Seizure, GTSC: Generalized Tonico-Clonic Seizure, SE: Status Epilepticus

	Mean onset latency (min)		
Treatment TSC (mg/kg)	PMS	GTCS	SE
0	-	-	-
7.5	75±7***	-	-
10	104±15**	117±17	125±09**
20	80±7**	139±11**	147±11*

Values were expressed as mean ± SEM. ANOVA: *p<0.05, **p<0.01, ***p<0.001 vs control

Table 3: Effects of pretreatments with ASMs on the onset latencies (mean ± SEM) of seizures induced by 10 mg/kg of TSC. PMS: Partial Myoclonic Seizure; GTSC: Generalized Tonic-Clonic Seizure; SE: Status Epilepticus; DW: Distilled Water

		Mean onset latencies (min)		
Pre-treatment (mg/kg)	Treatment ($TSC_{10\ mg/kg}$)	PMS	GTCS	SE
DW	+	104.0±15	117±7	125±9
PHB_{30}	+	-	-	-***
VPA_{200}	+	202.0±7^{**,b,cc,ddd}	240±8^{***,b,c,ddd}	-***
PNT_{200}	+	177.0±7^{**,aa,ddd}	220±8^{**,a,b,ddd}	-***
VPA_{100} + PNT_{15}	+	182.4±11^{***,aa,cc,ddd}	260±8^{***,a,c,ddd}	-***

Values were expressed as mean ± SEM. ANOVA: *p<0.05, **p<0.01, ***p<0.001 vs controls (DW); ^{a,b,c}p<0.05, ^{aa,cc}p<0.01, ^{ddd}p<0.001 vs drugs; ^{a}: VPA, ^{b}: VPA + PNT, ^{c}: PNT, ^{d}: PB

Table 4: Effects of pretreatments with psychopharmacologic drugs on onset latencies of the seizures induced by 10 mg/kg of TSC. PMS: Partial Myoclonic Seizure, GTCS: Generalized Tonic-Clonic Seizure, SE: Status Epilepticus, DW: Distilled Water

		Mean onset latencies (min)		
Pretreatment (mg/Kg)	TSC	PMS	GTCS	SE
DW	+	104±15	117±17	126±9
HAL_1	+	132±17	173±15*	217±11^{***,ccc}
$HAL_{1.5}$	+	78 ±12*	145±21*	181±18^{*,b,ccc}
CPZ_1	+	139±21	171±15*	218±9^{***,aa,ccc}
CPZ_2	+	144±17*	181±25*	221±27^{***,aa,ccc}
FXT_1	+	159±22*	193±13**	-^{***,aaa,bbb}
FXT_2	+	164.5±11*	173.5±8**	-^{***,aaa,bbb}

Values are expressed as mean ± SEM. ANOVA: *p<0.05, **p<0.01, ***p<0.001 vs controls (DW); ^{b}p<0.05, ^{aa}p<0.01, ^{aaa,bbb,ccc}p<0.001vs drugs; ^{a}: HAL, ^{b}: CPZ, ^{c}: FXT

All doses of HAL, CPZ, and FXT prolonged the onset latency of GTCS while HAL (1.5 mg/kg), CPZ (2 mg/kg), and FXT (1-2 mg/kg) prolonged the onset latency of PMS (Table 4). ANOVA with onset latency as a dependent variable and pharmacological treatment as an inter-subject factor revealed a significant effect of APDs and the SSRI on PMS (p<0.05) and on GTCS (p<0.05, p<0.01 respectively). Post-hoc pairwise comparisons revealed that the onset latency of GTCS differed significantly from that of PMS (p<0.01).

Discussion

The present behavioral and pharmacological study characterized the seizures induced by TSC in rats. Previous studies have shown the occurrence of TSC-induced epileptic discharges in cats (Dunlop *et al*., 1960), cerebral excitation in dogs (Roa *et al*., 1964), and seizures in mice (Golovenko *et al*., 2017). Due to the lack of studies on the rat and the difference in pharmacokinetic and metabolic properties between the rat and these species (Martignoni *et al*., 2006), it seemed useful to characterize these seizures in the rat. The use of the rat is important because rats and humans share an overwhelming majority of their biochemical capabilities at the genome level (Blais *et al*., 2017), and molecules susceptible to becoming drugs are routinely tested in rats to assess their safety and efficacy before clinical trials in humans.

TSC induced changes in the natural behavior of the rat. These changes were characterized by the appearance of freezing behavior, reduced locomotor activity, enhanced scratching, grooming, and exploratory behavior as well as the avoidance of central arena squares in the open field test. These changes in behavior could suggest an anxiety-like effect. We have indeed confirmed, by elevated plus maze experiments, the anxiogenic nature of the effects of TSC (results not shown).

The seizures induced by TSC have many characteristics in common with the seizures caused by drugs blocking GABAA receptors. The comparison of these seizures with the reported seizures induced by GABAA receptor

antagonists, PTZ, bicuculline, and picrotoxin (Velišek, 2009) showed that the clonic seizures lasted a few minutes while in the PTZ model, the duration of these seizures was several tens of seconds. In addition, the initial phase of GTCS, i.e., WR, was longer and more intense with TSC compared to PTZ. It is likely that the underlying mechanisms, which involve a different resultant action on GABA receptors, would be responsible for the observed behavioral differences. Indeed, while PTZ, bicuculline, or picrotoxin induce their effect by blocking GABAA receptors, TSC decreases the concentrations of GABA, which leads to the inactivation of GABAA and GABAB receptors. Therefore, it could be suggested that the increased severity was seen with TSC result of simultaneous inactivation of GABAA and GABAB receptors. The implication of the inactivation of GABAB receptors is attested by the fact that blockade of these receptors increased the frequency of spontaneous epileptiform discharges recorded extracellularly (Karlsson *et al*., 1992) and that their activation exerted a suppressing effect on different types of epileptiform discharges from hippocampal neurons. Furthermore, GABAB receptor agonists have been shown to diminish seizure activity in an animal model (Joshi *et al*., 2016).

A striking feature of the TSC-induced seizure model is the very long onset latency of seizures. The establishment of a very long delay could result from the pharmacokinetic properties of diffusion of the substance in different physiological compartments and through the BBB as well as from its mechanism of action on neuronal metabolism. A delay is necessary for the substance to diffuse toward the brain and for sufficient concentrations to accumulate in the neurons where the inhibition of GAD and consequently the inhibition of GABA synthesis could take place (Sze *et al*., 1971). The delay could also correspond, at least in part, to the time it would take for the conversion of TSC to a metabolite, which would be a more potent inhibitor of GAD, similar to what has been reported for another inhibitor of GAD, DL allylglycine (Horton, 1978). The observation that intracerebral microinjections of very low doses of TSC induced their effects with a differential latency of 10 min compared to GABAA antagonists microinjected into the same sites (Bagri *et al*., 2007) indicates the time required for the action on cell metabolism to take.

The very long latency property makes the use of the TSC model in the routine screening of potentially antiseizure molecules time-consuming compared to MES and PTZ. However, its utility could be in identifying potential anticonvulsant molecules that would not be effective in these large-scale screening models. An argument in favor of such a hypothesis is the fact that a category of molecules, the lead of which is levetiracetam, without an anticonvulsant effect observed in the MES and PTZ tests, was effective against seizures in chronic animal models such as kindled and genetic animal models (Gower *et al*., 1995). The model of seizures induced by TSC could also contribute to the elucidation of the pathophysiological mechanisms underlying the development of seizures and epilepsy. This is by modeling the decrease in GABA levels in the synaptic cleft as well as the interaction with glutamate metabolism (Collins, 1973) and the severe generalized convulsions these mechanisms may cause.

Regarding the neural network of seizures induced by TSC, induction of susceptibility to AGS with a low dose indicates that its neural network was activated at the same time as that of PMS. This well-identified network consisted mainly of the inferior colliculus and the pontine reticular formation (Bagri *et al*., 1989b; Faingold, 2009). As the dose of TSC increases, these brainstem structures and activated limbic structures during PMS can activate upper brain regions through their bilateral upward projections causing GTCS. The induced GTCS would therefore be considered as secondarily generalized rather than primarily generalized. This suggestion is supported by the more prolonged delay of GTCS than that of PMS since the propagation of epileptic activity to higher brain structures is necessary for the occurrence of GTCS while a lowering of the threshold of epileptic activity is necessary for the onset of PMS (Velišek, 2009).

High doses of TSC-induced SE end in death if not treated with diazepam or phenobarbital (results not shown). To our knowledge, this is the first time that SE has been described following TSC treatment in rats. SE consisted of prolonged seizures lasting more than 8 min, as defined for SE in humans. Analysis of its behavioral characteristics and pharmacological properties showed similarities with human pathology and other animal models. The seizure did not stop spontaneously when its duration exceeded 5-7 min and benzodiazepines were less effective than in GTCS. Diazepam decreased the severity but did not stop the seizures in a manner similar to what has been observed in humans and in Li/pilocarpine-induced SE. This reduced sensitivity of SE to benzodiazepines could have as a possible mechanism the decreased sensitivity to diazepam and Zn^{2+} of the GABA receptor currents while the effectiveness of PB could be linked to a preserved sensitivity of the GABA receptor currents to pentobarbital and GABA (Kapur, 2009).

TSC-induced SE may be related to the metabolic action of TSC at the cellular level and the consequent neuronal activation it induces. TSC is known to decrease the amount of GABA by inhibiting GAD presynapticaly, but it remains to be verified whether it also causes postsynaptic internalization of GABAA receptors. Thus, SE could result from a direct action of TSC via the reduction of the central amount of GABA or indirectly via the triggered seizures inducing internalization of GABAA receptors (Goodkin *et al*., 2008). The loss of GABA-mediated inhibition has been shown by the marked reduction in GABA receptor currents in CA1 pyramidal neurons (Kapur, 2009). As the rate of internalization of

GABAA receptors has been shown to be regulated by neuronal activity and its acceleration contributes to the reduction of inhibitory transmission observed during SE, it could be hypothesized that TSC, which induces neuronal activation, could, therefore, induce the internalization of GABAA receptors.

In the present study, ASMs prevented significantly both PMS and GTCS elicited by 10 mg/kg of TSC. The similarity of this pharmacological reactivity with ASMs treatment effects in human pathology and in other experimental seizure models is an additional argument validating TSC-induced seizures as a model of partial and secondary generalized tonic-clonic seizures. The mechanisms of action of the used ASMs could explain some differences in their efficacy. PB prevented completely PMS, GTCS, and SE. This strong efficacy could directly be linked to the fact that PB positively modulates every GABAA receptors subunits combination (Simeone and Rho, 2009). Therefore, PB potentiating effect may compensate for the reduction of GABA concentration in the synaptic cleft. VPA and PNT are also efficient but they act via common and different action mechanisms. VPA and PNT inhibit voltage-gated sodium channels and block sustained, high-frequency, repetitive firing neurons. PNT also inhibits the A-type potassium channel by increasing the probability of inactivated state (Simeone and Rho, 2009) and VPA antagonize glutamate AMPA receptors (Löscher and Klein, 2021). The similarity in the pharmacological treatment of TSC-induced seizures and seizure therapies in humans as well as in well-validated experimental models also relates to the establishment of synergy between ASMs. Indeed, a combination of low doses of VPA and PNT, which had no noticeable effect when used separately (results not shown), resulted in a significant reduction in the incidence and severity of PMS and GTCS. This result reflects the occurrence of synergy between VPA and PNT in the TSC model similar to the synergistic actions between different ASMs shown in an in vitro seizure model (Taing *et al*., 2017).

The results concerning the incidence of seizures clearly confirmed that the three PPDs tested possess anticonvulsant properties with varying intensities. FXT and CPZ were very effective, with FXT being superior at similar doses, while HAL had a weak effect. Regarding onset latencies, all tested doses of PPDs were found to delay latencies to PMS, GTCS, and SE. This could be related to the increase in the threshold of induction of PMS and to the decrease in the spread of seizures, necessary for the onset of GTCS. The effect of the two APDs on the seizures induced by TSC contrasted sharply with what is known in clinical practice and therefore traces the limit to the transposition of this model in human pathology. However, when we compare our results to what was shown in experimental models, some similarities are noted. Results with CPZ were similar to some data obtained in experimental models in rats such as maximal electroshock (Raines *et al*., 1976) and PTZ (Kim *et al*., 2003). For HAL, it was not possible to make a comparison because we could not find data obtained in rats. In a study carried out on mice, the absence of a protective effect on seizures induced by PTZ was observed (Ögren and Pakh, 1993). It seems that the variability of the effect of APDs is dependent on dose (Kretzschmar and Teschendorf, 1980) and age (Vernadakis, 1970). Interestingly, the doses of APDs used in the present study were very low compared to the doses used in the previous studies and the tested rats were young. Therefore, it would be necessary to conduct more experimental studies in different seizure models taking into account both age and dose parameters.

Regarding the effect of the antidepressant SSRI, FXT, our results were consistent with clinical and experimental data, which had shown a clear anticonvulsant effect. FXT was very effective against TSC-induced seizures in rats. This property is similar to what has been shown in other models of seizures in rats such as electroshock (Gangopadhyay *et al*., 2017) and AGS in GEPR (Yan *et al*., 1994). FXT may have multiple actions on neurotransmitter balance to prevent the initiation and spread of seizures induced by TSC. First, FXT can induce its anticonvulsant action in part by action on increasing serotonin in the synaptic cleft (Yan *et al*., 1994) by inhibiting serotonin reuptake (Fuller and Wong, 1977). This is also supported on the one hand by the fact that the increase in exogenous hippocampal serotonin produced anticonvulsant effects in the focal pilocarpine model for limbic psychomotor seizures and on the other hand, that citalopram, another SSRI, has shown an anticonvulsant effect in this model (Voskuyl and Klinckers, 2009). Second, the other mechanism that could contribute to the anticonvulsant action of FXT is its property of modulating GABAA receptors (Robinson *et al*., 2003; Walia and Gilhotra, 2017) giving this drug an anticonvulsant activity similar to potentiating drugs of GABAergic transmission. In addition, FXT is believed to have properties similar to those of SSRIs which cause an acute increase in GABA levels in the brain (Bhagwagar *et al*., 2004) as well as in GABAergic transmission from the hippocampus (Choi *et al*., 2010). Third, FXT reduces glutamate levels in the prefrontal cortex and thalamus (Kamal, 2010), which may also contribute to its anticonvulsant action.

Conclusion

The present study validated TSC-induced seizures in rats as a dose-dependent model of PMS, GTCS, and SE. PMS was elicited by a low dose while GTCS was elicited by a medium dose. A high dose of TSC always induced SE. TSC-induced seizures showed a very long onset latency of seizures. TSC-induced seizures have similar characteristics to GABAA receptor antagonists-induced seizures but with increased severity. ASMs (PHB, VPA, and PNT) and PPDs (HAL, CPZ, and FXT) prevented PMS and GTCS elicited

by TSC and prolonged their onset latencies. APDs also inhibited TSC-induced seizures in a way similar to what was observed in experimental models but not in clinical practice. FXT has an anticonvulsant effect similar to its effect in an animal model and in the human clinic.

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Author's Contributions

Anwar Satef: Experiments and data collection.

Sofia El Amoury: Data analyzed.

Soumaya El Ganouni and Abdelkhalid Essamadi: Review of the manuscript, correction.

Abdallah Bagri: Conception of the experiments, written of the manuscript.

Ethics

In this study, ethical principles related to scientific research were observed. The corresponding author confirms that the authors have read, revised, and approved the manuscript.

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