

Anticonvulsant Property of N-salicyloyltryptamine: Evidence of Enhance of Central GABAergic Neurotransmission

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ABSTRACT

Aim: In the present study we verified the anticonvulsant properties of the new tryptamine analogue, N-salicyloyltryptamine (NST), in rodents. **Methods and Results:** In the evaluation of the anticonvulsant activity, NST protected the animals from the incidence of seizures induced by pentylenetetrazole (PTZ) and picrotoxin (PIC), in doses of 100 and 200 mg/kg. NST (100 and 200 mg/kg, i.p.) significantly eliminated the extensor reflex of maximal electric-induced seizure tests in 40% of the experimental animals. However, in the PTZ model FLU (10 mg/kg, i.p.), an antagonist of the benzodiazepine (BZD) site in the GABA_A-BZD receptor complex, inhibited the prolongation of seizure latency induced by NST. **Conclusion:** Our results demonstrated an anticonvulsant activity of the new analogue that could be, at least in part, associated to the involvement of the GABAergic mechanism.

Key words: N-salicyloyltryptamine, anticonvulsant, PTZ, flumazenil, GABA_A.

RESUMO

Propriedade anticonvulsivante de N-saliciloiltryptamina: proteção significativa com o sistema GABAérgico

Objetivo: O presente estudo buscou avaliar o possível efeito anticonvulsivante do novo análogo da triptamina, N-saliciloiltryptamina (NST), em roedores. **Métodos e Resultados:** Na avaliação do efeito anticonvulsivante, os animais tratados com NST (100 e 200 mg/kg, i.p.) foram protegidos de maneira estatisticamente significativa ($p < 0,05$) quanto a latência e incidência do aparecimento das convulsões induzidas pela administração do pentilenotetrazol (PTZ) e da picrotoxina (PIC). O efeito protetor do NST nas convulsões induzidas pelo PTZ foi revertido pela administração do flumazenil (10 mg/kg, i.p.), um antagonista dos receptores GABA-benzodiazepínicos (GABA_A-BZD). A administração de NST (100 e 200 mg/kg, i.p.) protegeu de forma estatisticamente significativa ($p < 0,05$) os animais no teste das convulsões induzidas pelo eletrochoque-auricular em camundongos. **Conclusão:** Os resultados do presente estudo sugerem que o efeito anticonvulsivante de NST está associado, pelo menos em parte, ao sistema GABAérgico.

Unitermos: N-saliciloiltryptamina, anticonvulsivante, PTZ, flumazenil, GABA_A.

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INTRODUCTION

Epilepsy is the most common primary neurological disorder known, affecting 0.4-0.8% of the population and up to 50 million people worldwide.^{1,2} The term epilepsy is to some degree a misnomer, referring more accurately to a group of disorders with diverse origins and manifestations, collectively known as the epilepsies. The essential feature of the epilepsies is the appearance of behavioral changes, termed seizures.³ Seizures are classified according to their origins.⁴

The understanding of the pathophysiology of the epilepsies has advanced dramatically in the last 30 years, especially in terms of their cellular physiology and genetics. Drug treatment of epilepsy has also made remarkable strides, with the introduction of 11 new antiepileptic drugs (AEDs) since 1978: valproate, vigabatrin, tiagabine, lamotrigine, oxcarbazepine, felbamate, topiramate, gabapentin, levetiracetam, zonisamide, and pregabalin.⁵ Improvement in terms of clinical outcome, however, has fallen short of expectations, with up to one third of patients continuing to experience seizures or unacceptable medication-related side effects in spite of efforts to identify optimal treatment regimes with one or more drugs.⁶ On the other hand, there is still a need to develop new drugs with improved efficacy and tolerability for those patients which respond to current AEDs. Drugs preventing epilepsy or its progression would be an important innovation.⁷

N-Salicyloyltryptamine (NST) is a new analogue of *N*-benzoyltryptamine synthesized in our laboratory.⁸ Gutierrez et al.⁹ showed that *N*^b-benzoyltryptamine derivatives possess relaxant activity in guinea-pig ileum and Oliveira et al.¹⁰ attributed anticonvulsant properties. A preliminary behavioral screening in our laboratory with NST showed depressant effects on the CNS and anticonvulsant properties in mice.¹¹

The aim of this work was to investigate its possible anticonvulsant effect of the tryptamine analogue, *N*-salicyloyltryptamine (NST), in rodents.

MATERIAL AND METHODS

Animals

Male Swiss mice (25 ± 35 g) and male Wistar rats (250-300 g) were used. All of them were obtained from our research animal house and were maintained at a controlled room temperature ($21 \pm 2^\circ\text{C}$) with food and water *ad libitum*, as well as on a 12-h light/12-h dark cycle. All experiments were conducted between 08:00 and 17:00 h. Animals were previously habituated to the manipulations. Experimental protocols and procedures were approved by the Laboratório de Tecnologia Farmacêutica Animal Care and Use Committee (Nº 1105/06).

Drugs

Pentylentetrazole (PTZ), phenytoin (PHE), diazepam (DZP), flumazenil (FLU), and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma (USA). The NST was synthesized in our laboratory (LTF/UFPB/Brazil). In protocols *in vivo* the agents were injected intraperitoneally (i.p.) at a dose volume of 1 ml/10 g.

PTZ-induced seizures

The experiment used the method described by Goodman et al.¹² In such experiment, groups of mice ($n = 10$) were treated with NST (100 and 200 mg/kg, i.p.) or Tween 80 solution (0.2%), i.p. route, while the positive control was treated with diazepam (DZP, 2 mg/kg, i.p.). After 60 min of drug administration, the mice were treated with PTZ at a dose of 60 mg/kg (i.p.) and observed for at least 30 min to detect the occurrence of the first episode of forelimb clonus and the time before the onset of clonic seizures. The incidence of mortality was noted until 24 h after the injection of PTZ.

We also studied the effects of the selective GABA_A-BZD receptor antagonist, flumazenil (FLU, 10 mg/kg, i.p.),^{13,14} on the anticonvulsant activity of NST in order to investigate the probable involvement of GABA_A-BZD receptors. One group with ten mice received FLU 5 min before the administration of DZP (2 mg/kg, i.p.) (35 min before the injection of PTZ). The anticonvulsant activity of NST and DZP in mice pretreated with flumazenil was assessed and compared with the controls.

PIC-induced seizures

The method has been previously described.¹⁵ Animals were divided into five groups ($n = 10$). The first group served as control and received Tween 80 (0.2%) with one drop of cremophor, while the second group was treated with diazepam (DZP, 2 mg/kg i.p.). The remaining groups received an injection of NST, similar to the PTZ test. After 60 min of drug administration, the mice were treated with PIC at a dose of 8 mg/kg (i.p.). Immediately after the injection of the convulsant drug, mice were individually placed in plastic boxes and observed for the time onset of clonic seizures (latency), percent clonic seizures, and deaths. The incidence of deaths was noted until 48 h after the injection of PIC. DZP was used as the positive control.

Maximal electroshock test (MES)

MES produces reproducible tonic seizures characterized by tonic hindlimb extension (THE).¹¹ In this experiment, electroconvulsive shock (130 V, 150 pulses/s, 0.5 s) was delivered through auricular electrodes (ECT UNIT 7801-Ugo Basile) to induced THE. Mice were divided into five groups ($n = 10$). The first group served as the control and

received Tween 80 (0.2%) with one drop of cremophor, while the second group was treated with phenytoin (PHE, 30 mg/kg i.p.). The others groups received an injection of NST, similar to before the experiment. After 60 min all groups received electroconvulsive shock. The animals that did not exhibit THE were considered protected.¹⁶

Statistical analysis

Data obtained was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's *t* test. The incidence (%) of clonic or tonic-clonic seizures as well as mortality were evaluated by Fisher's Exact Test. Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

In the control group, PTZ consistently induced clonic seizures in 100% of 10 mice, with 80% mortality observed. Pretreatment with NST (100 and 200 mg/kg, i.p.) significantly reduced ($p < 0.05$) the incidence of clonic PTZ seizures and mortality. In addition, NST significantly increased the latency of clonic seizures at both tested doses. However, there was no significant difference between the latency of seizure in mice that had received NST (200 mg/kg) pretreated with FLU and the control group (Table 1).

Table 1. Effect of NST on PTZ-induced seizures in mice.

Treatment	Dose (mg/kg)	Latency (s) ^a	% Seizures	% Death
Control	–	161.3 ± 15.9	100	80
NST	100	351.7 ± 52.9 ^d	80 ^b	30 ^b
NST	200	343.9 ± 71.8 ^d	70 ^b	40 ^b
NST+FLU	200+10	213.8 ± 35.2	100	90
DZP	2	748.1 ± 38.9 ^e	10 ^c	0 ^c
DZP+FLU	2+10	238.6 ± 29.7	100	80

n = 10

^a Values represent mean ± S.D.

^b $p < 0.05$ (Fisher's test), significantly different from control.

^c $p < 0.01$ (Fisher's test), significantly different from control.

^d $p < 0.05$ (one-way ANOVA and Dunnett's test), significantly different from control.

^e $p < 0.01$ (one-way ANOVA and Dunnett's test), significantly different from control.

After receiving PIC (8 mg/kg i.p.) all mice of the control group exhibited convulsions. NST (100 and 200 mg/kg, i.p.) significantly increased the latency of the clonic seizures onset induced by PIC ($P < 0.05$) (Table 2).

As shown in Table 3, the treatment of mice with NST (100 and 200 mg/kg i.p.) significantly decreased the incidence of THE produced by MES. The highest dose of NST also completely protected the animals from lethality ($p < 0.05$, Fisher's test).

Table 2. Effect of the NST on PIC-induced seizures in mice.

Treatment	Dose (mg/kg)	Latency (s) ^a	% Seizures	% Death
Control	–	398.7 ± 28.1	100	90
NST	100	693.2 ± 41.8 ^d	80 ^b	60 ^b
NST	200	780.3 ± 68.7 ^d	60 ^c	60 ^c
DZP	2	1200.0 ± 0.0 ^e	0 ^c	0 ^c

n = 10

^a Values represent mean ± S.D.

^b $p < 0.05$ (Fisher's test), significantly different from control.

^c $p < 0.01$ (Fisher's test), significantly different from control.

^d $p < 0.05$ (one-way ANOVA and Dunnett's test), significantly different from control.

^e $p < 0.01$ (one-way ANOVA and Dunnett's test), significantly different from control.

Table 3. Effect of NST on MES induced tonic seizures in mice.

Treatment	Dose (mg/kg)	% tonic hindlimb seizures ^a	% Death
Control	–	100	90
NST	100	100	80
NST	200	70 ^b	60 ^b
PHE	30	10 ^c	0 ^c

n = 10

^a Values represent mean ± S.D.

^b $p < 0.05$ (Fisher's test), significantly different from control.

^c $p < 0.01$ (Fisher's test), significantly different from control.

DISCUSSION

The screening tests that have dominated the search for novel anticonvulsant drugs within pharmaceutical companies, academia and governmental agencies for the last half century have evolved from seizures induced by (a) maximal electroshock (MES) and (b) pentylenetetrazol (PTZ)⁽¹⁷⁾. Thus, the aim of this study was to assessment of the possible anticonvulsant effect of NST in PTZ-, PIC- and MES-induced convulsions on rodents.

When PTZ was administered, NST (100 and 200 mg/kg, i.p.) increased the latency time and reduced the death percentage significantly ($p < 0.05$). FLU (10 mg/kg, i.p.) inhibited the prolongation of seizure latency induced by NST (Table 1). All doses of NST significantly delayed ($p < 0.05$) the onset of seizures induced by PIC in mice (Table 2). In addition, NST (200 mg/kg, i.p.) significantly blocked seizures induced by MES ($p < 0.05$) (as shown in Table 3).

PTZ is considered to be an experimental model for the “generalized absence seizure”. PTZ may cause convulsions by inhibiting chloride ion channels associated with GABA_A receptors.^{18,19} Drugs that promote absence seizure or the increase of the latency in PTZ-induced seizures are suggested to possess anticonvulsant activity.²⁰ Benzodiazepines and many barbiturates act by promoting an increase of the synaptic inhibition interaction for

GABA, reducing the neuronal excitability and increased threshold seizures.⁷ Therefore, PIC has been shown to interact with the GABA neurotransmitter and the GABA receptor complex.^{7,19} In order to determine the role of BZD receptor participation in the NST-induced anticonvulsant effects, FLU, a specific antagonist of the benzodiazepine site in the GABA_A-BZD receptor complex,^{13,14,21} was used. The results obtained from the PTZ-induced seizure model in mice pretreated with FLU suggest that NST could facilitate the inhibitory activity of the GABAergic system probably through a competitive agonist action at the BZD site of the GABA receptors (Table 1 and 2). On the other hand, a study suggests anticonvulsant properties of NST in possible action of setorinergic system.¹¹

The maximal electroshock seizure test (MES), in which tonic hindlimb seizures are induced by bilateral corneal or transauricular electrical stimulation, is probably the best validated method for assessment of antiepileptic drugs in generalized tonic-clonic seizures ("grand mal").^{11,22} Several usual AEDs effective in the MES test protect against seizures through interactions with a variety of cellular targets, including modulation of voltage-dependent Na⁺ channels (phenytoin, carbamazepine, topiramate) or inhibition of intrinsic bursting (e.g. ethosuximide).²³

Furthermore, Araújo et al.²⁴ have demonstrated that NST promotes the blockade of sodium channels from GH3 cells, corroborating with the results of this work. However, the alteration produced by NST on the time constant of repolarization could also indicate a possible involvement of voltage-gated potassium channels, as was verified by Pisciotta and Prestipino²⁵ when fenitoin was used.

The present study provides evidence that NST has an anticonvulsant effect, although the precise mechanisms underlying the inhibitory effect of NST are not clear; however, its anticonvulsant property might involve a GABAergic mechanism.

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