ELSEVIER

Pharmacology, Biochemistry and Behavior 68 (2001) 199–202

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Anticonvulsant properties of *N*-salicyloyltryptamine in mice

Francisco A. Oliveira, Reinaldo N. de Almeida*, Maria de Fátima V. Sousa, José M. Barbosa-Filho, Silmara A. Diniz, Isac A. de Medeiros

Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, Caixa Postal 5009, CEP 58051-970, João Pessoa, PB, Brazil

Received 22 September 1999; received in revised form 30 August 2000; accepted 19 September 2000

Abstract

A new tryptamine analogue, *N*-salicyloyltryptamine (STP), a potential central nervous system (CNS) depressant, was tested in the pentylenetetrazol (PTZ) and maximal electroshock (MES) models of epilepsy in mice. When administered concurrently, STP (100 mg/kg ip) significantly reduced the number of animals that exhibited PTZ-induced seizures and eliminated the extensor reflex of maximal electric-induced seizures test in 50% of the experimental animals. In addition, it showed protection in the PTZ test by diminishing the death rate.

Keywords: Anticonvulsant activity; *N*-Salicyloyltryptamine; Pentylenetetrazol; Epilepsy; Maximal electroshock

1. Introduction

Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures. The various forms of epilepsy and epileptic syndromes are defined by several signs and symptoms that include characteristic types of seizures, other clinical features, and family history (Engel, 1995).

Current epidemiological studies show a prevalence rate for active epilepsy in 0.5–1% of the population (Hachinski, 1998). Several hypotheses have been advanced to explain the cause of primary or idiopathic epilepsy, including alterations in several classic neurotransmitter systems such as the glycine, glutamatergic, and GABAergic neurotransmitter systems. More recently, other molecules such as nitric oxide have been suggested as potential neurotransmitters or retrograde messengers (Del-Bel et al., 1997), but none is completely satisfactory.

An antiepileptic drug may be defined as a drug that, when administered over a prolonged period, will decrease the incidence and/or severity of seizures occurring in patients with epilepsy. These drugs are used mainly for suppression of epileptic seizures without impairment of the central nervous system (CNS) and without depression of respiration (Schlinger and Poling, 1988).

Antiepileptic drugs are found in several different chemical classes. Most of the drugs introduced before 1965 are closely related in structure to phenobarbital. These include hydantoins, deoxybarbiturates, and succinimides. More recent agents include benzodiazepines (clonazepam and clorazepate), an iminostilbene (carbamazepine), a carboxylic acid (valproic acid), felbamate, lamotrigine, gabapentin, topiramate, tiagabine, and vigabatrin (Blum, 1998; Chadwick, 1998; Shorvon and Stefan, 1997), while several new anticonvulsant drugs are currently being evaluated (Wieland et al., 1997; Thurggur and Church, 1998).

N-Salicyloyltryptamine (STP) is a new analogue of *N*-benzoyltryptamine (Hifnawy et al., 1957) synthesized in our laboratory. In a preliminary behavioral screening in our laboratory, STP showed depressant effects on the CNS in mice (Oliveira et al., 1996). Therefore, in the present work, we attempted to investigate the effect of STP in seizures induced by pentylenetetrazol (PTZ) and in maximal electroshock (MES).

2. Materials and methods

2.1. Animals

Male Swiss mice (25–35 g) were used. All of them were obtained from our research animal house and were

* Corresponding author. Tel.: +55-83-216-7375; fax: +55-83-216-7511.
E-mail address: reinaldo@ltf.ufpb.br (R.N. de Almeida).

0091-3057/01/$ – see front matter
PII: S0091-3057(00)00484-6

Table 1
Effect of 100 mg/kg ip dose of STP on PTZ-induced convulsive seizures in mice

Treatment	*n*	Dose (mg/kg)	Latency of clonic seizures (s)[a]	Percent clonic convulsions	Percent mortality
Vehicle + PTZ	10	–	180.4 ± 20.7	100	80
STP + PTZ	10	100	203.4 ± 16.4	60[b]	30[c]
DZP + PTZ	10	5	985.0 ± 35.3[d]	20[c]	0[c]

n: number of mice per group.
[a] Values represent mean ± S.D.
[b] $P < .05$ (Fisher's test), significantly different from control.
[c] $P \leq .001$ (Fisher's test), significantly different from control.
[d] $P < .05$ (one-way ANOVA and Dunnetts's test), significantly different from control.

maintained at a controlled room temperature (21 ± 2°C) with food and water ad libitum, as well as on a 12-h light/12-h dark cycle. All experiments were conducted between 0800 and 1700 h. Animals were previously habituated to the manipulations.

2.2. Drugs

PTZ, phenytoin, and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma (USA). The STP was synthesized in our laboratory (Brazil).

2.3. Convulsing tests

2.3.1. PTZ-induced convulsions

PTZ (60 mg/kg ip) was used to induce clonic convulsions (Swinyard et al., 1989). Mice were divided into three groups ($n = 10$). The first group served as control and received Tween 80 (0.2%), while the second group was treated with diazepam (DZP, 5 mg/kg ip). The remaining group received an injection of STP at a dose of 100 mg/kg. After 60 min of drug administration, the mice were treated with intraperitoneal PTZ at a dose of 60 mg/kg and observed for at least 30 min to detect the occurrence of the first episode of forelimb clonus. The incidence of mortality was noted until 24 h after the injection of PTZ.

2.3.2. Maximal electroshock

MES produces reproducible tonic convulsion characterized by tonic hindlimb extension (THE). In this experiment, electroconvulsive shock (130 V, 150 pulses/s, 0.5 s) was delivered through auricular electrodes (ECT UNIT 7801-Ugo Basile) to induce THE. Mice were divided into five groups ($n = 10$). The first group served as control and received only Tween 80 (0.2%), while the second group was treated with phenytoin (standard antiepileptic drug, 20 mg/kg ip). The three remaining groups received an injection of STP at doses of 50, 100, and 200 mg/kg. All the treatments were administered 60 min before the electrical stimulation. Immediately after electroconvulsive shock, the mice were placed in clear plastic boxes and observed for the occurrence of tonic convulsion and the latency. Animals that did not exhibit THE were considered protected (Tortoriello and Ortega, 1993).

2.3.3. Statistical analysis

The latencies to forelimb clonus and tonic convulsion were analyzed using a one-way ANOVA followed by Dunnett's *t* test. The incidence (%) of clonic or tonic convulsions as well as the mortality were evaluated by the Fisher's Exact Test. Differences were considered to be statistically significant when $P < .05$.

3. Results

This study gives preliminary evidence that acute treatment with STP reduces partially the incidence of seizures in two common models used for testing of anticonvulsant substances. However, this effect was less than that observed for the standard drugs, DZP, and phenytoin used in the PTZ and MES model, respectively.

3.1. Effects on PTZ-induced seizures

In the control group, PTZ consistently induced clonic seizures in 100% of 10 mice, with 80% mortality observed. Pretreatment with the STP at a dose of 100 mg/kg ip significantly reduced ($P < .05$) the incidence of clonic PTZ seizures and mortality. However, STP did not affect the latency of clonic seizures at the tested dose. On the other hand, the pretreatment with DZP significantly

Table 2
Effect of increasing doses of STP (50, 100, and 200 mg/kg ip) on MES-induced tonic seizures in mice

Treatment	*n*	Dose (mg/kg)	Percent tonic hindlimb convulsions[a]	Percent mortality
Vehicle	10	–	100	100
STP	10	50	100	60
STP	10	100	50[b]	20[b]
STP	10	200	40[b]	0[c]
Phenytoin	10	20	0[c]	0[c]

n: number of mice per group.
[a] Values represent percentage of mice that did present tonic convulsion.
[b] $P < .05$ (one-way ANOVA and Fisher's test), significantly different from control.
[c] $P < .001$ (one-way ANOVA and Fisher's test), significantly different from control.

prolonged the latencies and was effective in preventing clonic seizures induced by PTZ in 80% of the animals (Table 1).

3.2. Effect on MES-induced convulsions

As shown in Table 2, the treatment of mice with STP (100 and 200 mg/kg ip) significantly decreased the incidence of THE produced by MES. The highest dose of STP also completely protected the animals from lethality ($P < .01$, Fisher's test).

4. Discussion

The results of the present study show that acute administration of STP (100 mg/kg ip) on PTZ-induced convulsive seizures in mice significantly reduced ($P < .05$) the incidence of clonic seizures and mortality (as shown in Table 1).

In other anticonvulsant test, acute treatment with STP at 100 and 200 mg/kg inhibited 50% and 60% of tonic hindlimb convulsions induced by MES, respectively. These treatments also significantly reduced the mice mortality (Table 2).

The anticonvulsant profile shows that STP has positive activity in both tests, namely chemical and electrical tests. These two animal models are usually considered suitable for identification of antiseizure activity of all compounds currently used for the treatment of a wide variety of seizure types. They may predict clinical efficacy and the mechanism of drug action (Kupferberg, 1992; Wieland et al., 1997; Bourgeois, 1998).

The first model of seizure, PTZ-induced seizures, is considered as an experimental model for the "generalized absence seizure." It produces both clonic and tonic seizures when administered parenterally. A progression of symptoms is seen, depending on the dose and the route of administration. These symptoms follow a pattern of focal activation of the CNS, such as myoclonic jerks and clonic spasms, then a generalized tonic seizure. The other methodology employed, the MES test, is the most frequently used as an animal model for identification of anticonvulsant activity of drugs for the "grand mal."

Although various mechanisms of anticonvulsant action have been proposed, none enjoys general acceptance, including the increase of the concentration of biogenic amines. It is based on the fact that various anticonvulsants, by nonspecific depression of CNS function, increase the levels of serotonin in the brain (Korolkovas and Burckhalter, 1976; Dayley et al., 1996; Meshkibaf et al., 1995; Southam et al., 1998). In addition, certain serotonin biosynthetic precursors are effective in epilepsy treatment (Reynolds and Shorvon, 1981; Shandra et al., 1998). Also, reuptake inhibitors such as fluoxetine have been shown to exert an anticonvulsant effect in several animal models of epilepsy by inducing an increase in extracellular serotonin (Yan et al., 1994). The 5-HT1A receptor antagonist (−)-pindolol and LY 206130 (1-[1-*H*-indol-4-yloxy]-3-[cyclohexylamino]-2-propanol maleate), when administered in combination with fluoxetine, produced a marked potentiation of the anticonvulsant effect in severe seizure of genetically epilepsy-prone rats (Browning et al., 1997). In another study, it was observed that valproate substantially reduced the concentrations of homovanillic acid and hydroxyindolacetic acid, metabolites of dopamine and 5-HT (Vriend and Alexiuk, 1996).

The present study provides evidence that STP has an anticonvulsant effect, although the precise mechanisms underlying the inhibitory effect of STP are not clear; however, their antiseizure effects might involve serotonergic mechanisms, since it is structurally similar to serotonergic drugs.

Although STP is but modestly effective when compared to the standard drugs, DZP and phenytoin in PTZ and MES models, the effects observed for this compound appear to offer a potential advantage over any anticonvulsant drug since these are only effective in one of these models. For example, DZP is especially effective in preventing the clonic convulsions induced by PTZ but does not block generalized clonic–tonic convulsions induced by MES. In contrast, the most significant effect of phenytoin is its ability to modify the pattern of MES seizures. The characteristic tonic phase can be abolished completely, but clonic seizures may be increased and prolonged.

Acknowledgments

The authors would like to express their sincere thanks to Prof. Joseph Miller for manuscript revision and J.C. Duarte for his technical assistance. This work was supported by CNPq and CAPES of Brazil.

References

Blum DE. New drugs for persons with epilepsy. Adv Neurol 1998;76:57.

Bourgeois BFD. New antiepileptic drugs. Arch Neurol 1998;55:1181.

Browning RA, Wood AV, Merrill MA, Dailey JW, Jobe PC. Enhancement of the anticonvulsant effect of fluoxetine following blockade of 5-HT1A receptors. Eur J Pharmacol 1997;336:1.

Chadwick D. Do new antiepileptic drugs justify their expense? Arch Neurol 1998;55:1140.

Dayley JW, Yan QS, Adams-Curtis LE, Ryu JR, Ko KH, Mishra PK, Jobe PC. Neurochemical correlates of antiepileptic drugs in the genetically epilepsy-prone rats (GEPR). Life Sci 1996;58(4):259.

Del-Bel EA, Oliveira PR, Oliveira JAC, Mishra PK, Jobe PC, Garcia-Cairasco N. Anticonvulsant and proconvulsant roles of nitric oxide in experimental epilepsy models. Braz J Med Biol Res 1997;30:971.

Engel J. Concepts of epilepsy. Epilepsia 1995;36(1):23.

Hachinski V. New antiepileptic drugs — the cost of innovation. Arch Neurol 1998;55:1142.

Hifnawy MS, Vaquette J, Sévenet T, Pousset JL, Cavé A. Produits neutres et alcaloides de *Myrtopsis macrocarpa*, *M. myrtoidea*, *M. novae-caledoniae* et *M. sellingii*. Phytochemistry 1957;16:1035.

Korolkovas A, Burckhalter JH. Anticonvulsants. Essential of medicinal chemistry. New York: Wiley, 1976. p. 105.

Kupferberg HJ. Strategies for identifying and developing new anticonvulsant drugs. Pharm Weekbl 1992;14:132.

Meshkibaf MH, Subhash MN, Lakshmana KM, Rao BS. Effect of chronic administration of phenytoin on regional monoamine levels in rat brain. Neurochem Res 1995;20:773.

Oliveira FA, Souza MFV, Almeida RN. Estudo da atividade central dos alcalóides totais de *Rauwolfia ligustrina* Roem and Schultt (Mata-pasto) e substâncias indólicas. XIV Simp Plant Med Bras (Abstr) 1996;14:155.

Reynolds EH, Shorvon SD. Nontherapy or polytherapy for epilepsia? Epilepsia 1981;22:10.

Schlinger H, Poling A. Acute and chronic effects of methsuximide and mephenytoin on delayed-matching-to-performance of pigeons. Psychopharmacology 1988;95:82.

Shandra AA, Godlevskii LS, Brusentsov AI, Petrashevich VP, Vast'Yanov RS, Nikel B, Mikhaleva II. Delta-sleep-inducing peptide and its analogs and the serotoninergic system in the development of anticonvulsive influences. Neurosci Behav Physiol 1998;28:521.

Shorvon S, Stefan H. Overview of the safety of newer antiepileptic drugs. Epilepsia 1997;38:S45.

Southam E, Kirkby D, Higgins GA, Hagan RM. Lamotrigine inhibits monoamine uptake in vitro and modulates 5-hydroxytryptamine uptake in rats. Eur J Pharmacol 1998;358:19.

Swinyard EA, Woodhead JH, White HS, Franklin MR. Experimental selection, quantification and evaluation of anticonvulsants. In: Levy RH, Dreyfuss FE, Mattson RM, Meldrum BS, Penry JK, editors. Antiepileptic drugs. New York: Raven Press, 1989. p. 85.

Thurggur C, Church J. The anticonvulsant action of σ receptor ligands in the Mg^{2+}-free model of epileptiform activity in rat hippocampal slices. Br J Pharmacol 1998;124:917.

Tortoriello J, Ortega A. Sedative effect of galphimine B, a Nor-seco-triterpenoid from *Galphimia glauca*. Planta Med 1993;59:398.

Vriend JP, Alexiuk NA. Effect of valproate on amino acid and monoamine concentrations in striatum of audiogenic seizure-prone Balb/c mice. Mol Chem Neuropathol 1996;27:307.

Wieland S, Belluzzi J, Hawkinson JE, Hogenkamp D, Upasani R, Stein L, Wood PL, Gee KW, Lan NC. Anxiolytic and anticonvulsant activity of a synthetic neuroactive steroid Co 3-0593. Psychopharmacology 1997;134:46.

Yan QS, Jobe PC, Cheong JH, Ko KH, Dailey JW. Role of serotonin in the anticonvulsant effect of fluoxetine in genetically epilepsy-prone rats. Naunyn-Schmiedeberg's Arch Pharmacol 1994;350:149.