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Research Article

Bioassay-Guided Evaluation of Antinociceptive Effect of *N*-Salicyloyltryptamine: A Behavioral and Electrophysiological Approach

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We investigated the antinociceptive and nerve excitability effects of the *N*-salicyloyltryptamine (NST) NST-treated mice exhibited a significant decrease in the number of writhes when 100 and 200 mg/kg (i.p.) were administered (i.p.). This effect was not antagonized by naloxone (1.5 mg/kg, i.p.). NST inhibited the licking response of the injected paw when 100 and 200 mg/kg were administered (i.p.) to mice in the first and second phases of the formalin test. Because the antinociceptive effects could be associated with neuronal excitability inhibition, we performed the single sucrose gap technique and showed that NST (3.57 mM) significantly reduced (29.2%) amplitude of the compound action potential (CAP) suggesting a sodium channel effect induced by NST. Our results demonstrated an antinociceptive activity of the NST that could be, at least in part, associated to the reduction of the action potential amplitude. NST might represent an important tool for pain management.

1. Background

In the past few decades, a number of studies have been performed in an attempt to elucidate and understand the factors and mechanisms involved in normal sensory, pain perception and its treatment. However, although a considerable number of analgesic drugs are available for pain management, the search for the development of new compounds as therapeutic alternatives continues as those drugs present a wide range of side effects [1].

Furthermore, Davies et al. [2] have reported the use of anticonvulsant and antidepressive drugs in the treatment of chronic neuropathic or inflammatory pain. The anticonvulsant drugs are able to reverse or avoid seizures acting by decreasing the neuronal response to seizure-induced stimuli which are commonly used to treat pain disorders [3]. These drugs have a role in its treatment due to the fact that some clinical pain disorders seem to have common physiopathogenic mechanisms with seizures [4]. In this way, phenytoin and carbamazepine have been evidenced to exert

an analgesic effect on trigeminal neuralgia, glossopharyngeal and occipital neuralgia [5, 6], and diabetic neuropathy [2].

N-Salicyloyltryptamine (NST) is a new analogue of *N*-benzoyltryptamine synthesized in our laboratory (Patent claimed, BR 200304393-A, [7]). Gutierrez et al. [8] showed that N^b-benzoyltryptamine derivatives have relaxant activity in guinea-pig ileum and Oliveira et al. [9] attributed anticonvulsant properties to it. In preliminary behavioral screening with NST, Oliveira et al. [10] showed a depressant effect on the CNS and anticonvulsant property in mice.

Thus, the aim of this study was to verify the antinociceptive effect of *N*-salicyloyltryptamine (NST), the tryptamine analogue, using different experimental models of nociception, as well as to investigate whether such effect might be involved in the nerve excitability through the single sucrose gap technique.

2. Methods

2.1. Animals. Male Swiss mice (25–32 g) and male Wistar rats (250–300 g) were used. All of them were obtained from the Laboratory of Pharmaceutical Technology Animal Care and were 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 8 AM and 5 PM. Animals were previously habituated to the manipulations. Experimental protocols and procedures were approved by the Laboratory of Pharmaceutical Technology Animal Care and Committee (no. 1105/06).

2.2. Drugs. For all *in vivo* experiments, the following drugs were used: NST (Laboratory of Pharmaceutical Technology, Brazil) (Figure 1), morphine hydrochloride (Cristália, Brazil), indomethacin (Sigma, USA), naloxone hydrochloride (Neoquímica, Brazil), 37% formaldehyde (Vetec, Brazil), and acetic acid (Vetec, Brazil). Vehicle was 5% Tween 80 (Sigma, USA) dissolved in 0.9% saline solution and used to dilute the test drugs. In *in vivo* protocols, the agents were injected intraperitoneally (i.p.) at a dose volume of 1 mL/10 g (mice). The physiologic solution used for the *in vitro* tests was composed by (in mM): NaCl 150; KCl 4; $CaCl_2$ 2; $MgCl_2$ 1; glucose 10; [N-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid] (HEPES) 10, adjusted to pH 7.4 with NaOH.

2.3. Acetic Acid-Induced Writhing. Initially, the mice were divided into five groups ($n = 10$, each). Then, they were pretreated with NST (100 and 200 mg/kg, i.p.) or tween 80 solution (0.2%, i.p.), while positive control was treated with morphine (5 mg/kg, i.p.) 45 min before an injection of 0.85% acetic acid (0.25 ml/animal, i.p.). The doses of NST applied in the present study were based on the study of Oliveira et al. [10]. Each animal was isolated in an individual observation chamber and 10 min after acetic acid injection, the cumulative number of writhing responses was recorded during 15 min [1, 11]. The effect of pretreatment with naloxone (1.5 mg/kg, i.p., 30 min before the algogen administration) on the antinociception produced by NST (100 and 200 mg/kg) and morphine (5 mg/kg, i.p.) was also determined. An additional group pretreated with vehicle received a similar volume of NST vehicle (tween 80 solution 0.2%).

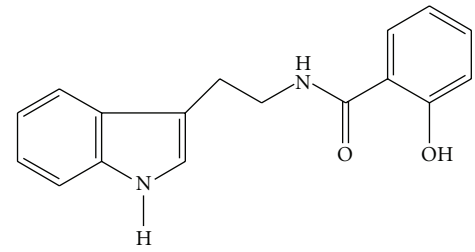

Figure 1: Molecular structure of the *N*-salicyloyltryptamine.

2.4. Formalin-Induced Nociception. The formalin test was carried out as described by Hunskaar and Hole [12]. Mice were divided into five groups ($n = 10$, each) and treated i.p. with vehicle (control), NST (100 and 200 m/kg, i.p.), and indomethacin (10 mg/kg, i.p.). After 45 min, 20 μL of 2.5% formalin solution (0.92% formaldehyde in 0.9% saline) was injected into the subplantar area of the right hindpaw. The duration of paw licking was measured at 0–5 min (first phase) and 15–30 min (second phase) after formalin administration.

2.5. Rota-Rod Test. Initially, the mice able to remain on the Rota-rod (AVS, Brazil) longer than 210 s (9 rpm) were selected 12 and 24 h before the test [13, 14]. Then, the selected animals were divided into four groups ($n = 10$) and treated i.p. with vehicle (control), NST (100 and 200 ml/kg, i.p.), and diazepam (3 mg/kg, i.p,). Thirty minutes later, each animal was tested on the Rota-rod and the time(s) they remained on the bar for up to 210 s was recorded after 30, 60, and 90 min.

2.6. Electrophysiologic Assays. Procedures for isolated nerve experiments were fundamentally the same as described in previous papers [15, 16]. In short, the sciatic nerves of rats (*Rattus norvegicus*) were carefully removed and desheathed. One nerve bundle was positioned across the five compartments of the experimental chamber, which contained Vaseline at the partitions to electrically isolate them. Compartments 1 and 2, at one end of the nerve bundle, were used to apply supramaximal stimulation, which consisted of 100 μs isolated rectangular voltage pulses (4–6 V), delivered by a stimulator (CF Palmer, Model 8048, UK), triggered manually. These parameters were chosen to selectively stimulate fast-conducting myelinated fibers (Aα). All compartments were filled with physiological solution with the following composition (in mM): NaCl 150; KCl 4.0; $CaCl_2$ 2.0; $MgCl_2$ 1.0; [*N*-(2-hydroxyethyl) piperazine-*N*′-2-ethanesulfonic acid] (HEPES) 10, adjusted to pH 7.4 with NaOH, except for the fourth compartment, which was filled with isotonic (280 mM) sucrose solution that was continuously renewed to electrically isolate the neighboring recording compartments. The NST at concentration of

3.57 mM was introduced into the test (central) compartment. This concentration was chosen by the concentration-response curve (data not shown). The potential difference between the test and the fifth (last) compartment was recorded every 10 min. Data were converted to digital form by a microcomputer-based 12-bit A/D converter at a rate of 10.5 kHz and later analyzed using a suite of programs (Lynux, São Paulo, Brazil). To quantify the effects of NST, we used the amplitude (which is the potential difference between the baseline and the maximal voltage of the compound action potential—CAP), and the time constant of repolarization (τ) that was calculated by the equation $V = V_0^* \exp(t/\tau)$ using nonlinear regression analysis applied to the repolarization phase of the compound action potential (CAP). All experiments were conducted at room temperature ($21 \pm 2^\circ$C).

2.7. Statistical Analysis.

In vivo test. The data obtained were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t or Fisher's Exact Tests.

In vitro test. For CAP recordings, the significant level was obtained using the two-tailed Student's t-test. Differences were considered to be statistically significant when $P < .05$.

The percent of inhibition caused by an antinociceptive agent was determined for the acetic acid-induced writhing and formalin tests through the following formula [17]:

$$\text{Inhibition } \% = 100 \cdot \frac{(\text{control} - \text{experiment})}{\text{control}}. \quad (1)$$

3. Results

In the acetic acid-induced writhing test, the antinociceptive effect, represented by writhe reduction, elicited by 100 and 200 mg/kg of NST (14.1 ± 7.2 and 12.9 ± 6.3) in mice. Morphine (5 mg/kg) (1.3 ± 0.6) produced a reduction of nociceptive response. In contrast, naloxone (1.5 mg/kg, i.p.), an opioid antagonist, showed no influence on the antinociceptive action of NST (100 and 200 mg/kg, i.p.) (Table 1). The group that received only the diluent of NST (tween 80 solution 0.2%) did not present significant behavioral alteration (data not shown).

Effects of NST on formalin test are shown in Table 2. All test groups are significantly different from control group on the early and late phases ($P < .01$). NST (100 and 200 mg/kg, i.p.) caused graded inhibition of both phases of formalin-induced pain.

As shown in Table 3, the treatment of mice with NST (100 and 200 mg/kg, i.p.) did not show significant motor performance alterations (Table 3).

Figure 2 illustrates a typical example of the effects of NST (3.57 mM) on the CAP that was incubated at 30 min showing decreased amplitude after NST incubation. This incubation time was sufficient once that from 20 min of NST addition, we verified a stabilized effect on the CAP amplitude. That amplitude reduction produced by NST is shown in Figure 3.

Table 1: Effect of NST or morphine on acetic acid-induced writhing.

Treatment	Dose (mg/kg)	Number of writhings[a]	% Inhibition
Vehicle	—	39.4 ± 7.9	—
NST	100	14.1 ± 7.2[c]	64.2[e]
NST	200	12.9 ± 6.3[c]	67.3[e]
NST + naloxone	100 + 1.5	21.8 ± 7.7[b]	44.7[e]
NST + naloxone	200 + 1.5	18.1 ± 9.4[b]	50.1[e]
Morphine	5	1.3 ± 0.6[d]	96.7[f]
Morphine + naloxone	5 + 1.5	35.2 ± 5.8	10.7

$n = 10$ per group.
[a]Values represent mean ± S.E.M.
[b]$P < .05$ (one-way ANOVA and Dunnett's test), significantly different from control.
[c]$P < .01$ (one-way ANOVA and Dunnett's test), significantly different from control.
[d]$P < .001$ (one-way ANOVA and Dunnett's test), significantly different from control.
[e]$P < .01$ (Fisher's test), significantly different from control.
[f]$P < .001$ (Fisher's test), significantly different from control.

Table 2: Effect of NST or indomethacin on formalin-induced pain.

Treatment	Time of nociceptive behavior (s)			
	0–5 min		15–30 min	
	Score of pain[a]	% inhibition	Score of pain[a]	% inhibition
Vehicle	92.3 ± 24.4	—	124.2 ± 23.1	—
NST (100 mg/kg)	43.8 ± 16.1[b]	52.5[d]	58.1 ± 17.3[b]	53.2[d]
NST (200 mg/kg)	39.9 ± 21.7[b]	56.8[d]	46.4 ± 13.2[a]	62.6[d]
Indomethacin (10 mg/kg)	78.4 ± 18.9	15.1	14.9 ± 8.9[c]	88.0[e]

$n = 10$ per group.
[a]Values represent mean [total time spent in licking (s)] ± S.E.M.
[b]$P < .05$ (one-way ANOVA and Dunnett's test), significantly different from control.
[c]$P < .001$ (one-way ANOVA and Dunnett's test), significantly different from control.
[d]$P < .01$ (Fisher's test), significantly different from control.
[e]$P < .001$ (Fisher's test), significantly different from control.

Table 3: Effect of NST or diazepam on the Rota-rod test.

Treatment	Time (s) on Rota-rod/210 s[a]		
	30 min	60 min	90 min
Vehicle	210.0 ± 0.0	195.3 ± 4.8	210.0 ± 0.0
NST (100 mg/kg)	189.9 ± 5.1	181.5 ± 7.4	210.0 ± 0.0
NST (200 mg/kg)	195.3 ± 5.8	210.0 ± 0.0	179.8 ± 9.3
Diazepam (3 mg/kg)	38.7 ± 9.7[b]	17.2 ± 11.3[b]	23.5 ± 15.9[b]

The motor response was recorded for the following 210 s after drug treatment, $n = 10$, [a]Values represent mean ± S.E.M., [b]$P < .01$ (one-way ANOVA and Dunnett's test), significantly different from control.

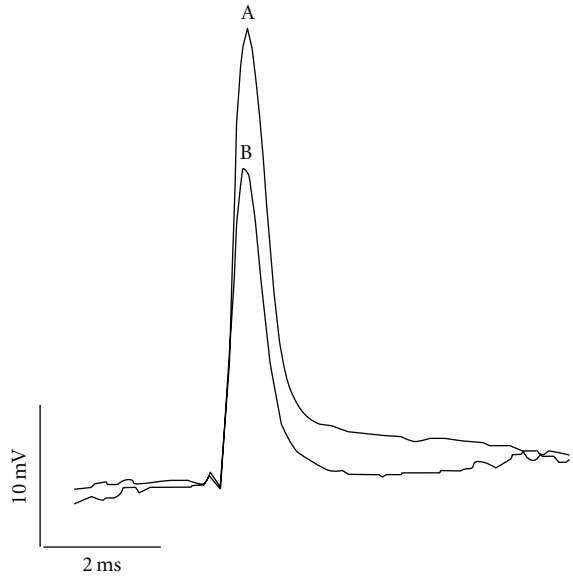

Figure 2: Effect of NST on nerve compound action potential (CAP). The figure shows representative superimposed CAP recordings, in the absence (control, A) and 30 min after the addition of NST (3.57 mM) (B).

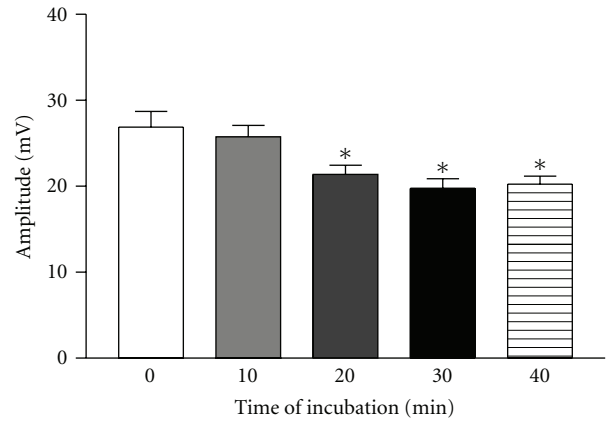

Figure 3: Effect of NST (3.57 mM) on the CAP amplitude. This figure shows the time-course of NST incubation. The bars indicate the mean ± S.E.M. of 5 experiments. $^*P < .05$ in relation to absence of NST addition.

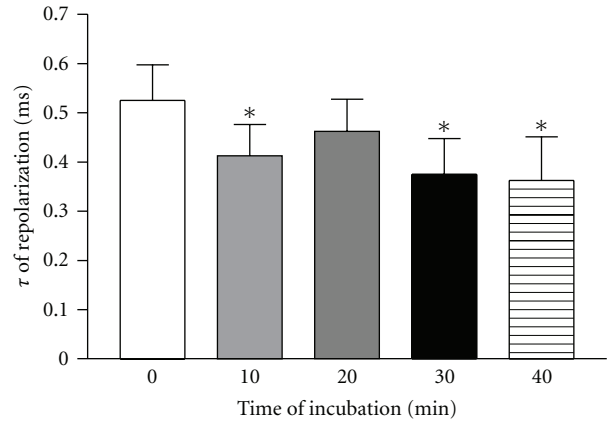

Figure 4: Effect of NST (3.57 mM) on the time constant of CAP repolarization. This figure shows the time-course of NST incubation. The bars indicate the mean ± S.E.M. of 5 experiments. $^*P < .05$ in relation to absence of NST addition.

Figure 4 shows the time constant of CAP repolarization (τ_{CAP}) and during NST (3.57 mM) incubation at 10, 30, and 40 min the τ_{CAP} was decreased, showing a time-dependent effect that was stabilized after 20 min of NST addition.

4. Discussion

In the past years, the discovery and development of central analgesic drugs, non-opioid system, has been one of the noticeable research fields. However, most antiepileptic drugs currently used in therapy of epilepsy exhibit analgesic effect and are used in the treatment of chronic neuropathic pain (trigeminal pain). The main goal of this study was to evaluate the possible antinociceptive effect of the NST in rodents and in sucrose-gap technique.

In the writhing test, doses of 100 and 200 mg/kg of NST significantly reduced the number of writhing movements induced by the i.p. administration of the acetic acid solution (Table 1). This result indicates that the NST has antinociceptive activity, but this test is nonspecific to determine this kind of activity, even though it can be widely used for analgesic screening [18]. It was observed that naloxone (1.5 mg/kg, i.p.) antagonized the antinociceptive response of morphine (standard drug). However, naloxone did not reverse the effect of NST (100 and 200 mg/kg, i.p.) (Table 1). That suggests the nonparticipation of the opioid system in the modulation of pain provoked by NST.

The formalin test is a valid and reliable model of nociception and it is sensitive for several classes of analgesic drugs. Formalin test produced a distinct biphasic response and different analgesics may act differently in the first and second phases of this test. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic [19]. Centrally acting drugs such as opioids inhibit both phases equally [20], but peripherally acting drugs such as aspirin, indomethacin, and dexamethasone only inhibit the late phase [12]. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [12, 13]. The effect of NST on the first and second phases (Table 2) of formalin test suggests that its activity may result from its central action, but without the opioid system participation.

Previous studies suggested that the CNS depression and the nonspecific muscle relaxation effect can reduce the response of motor coordination and, consequently, might invalidate the formalin test results [13, 14]. As Oliveira et al. [10] showed depressant effect on the CNS in mice treated with NST, we realized motor coordination test (Rota-rod). Our results demonstrated that NST-treated (100 and 200 mg/kg, i.p.) mice showed no performance alterations in the Rota-rod test with the doses used (Table 3). We did not see any motor coordination change for the animals,

therefore, eliminating a nonspecific muscle relaxation effect of NST at the doses used.

In this study, the reduction of CAP amplitude induced by NST suggests a voltage-gated sodium channel blocker. Some anticonvulsant agents, used in trigeminal pain, have shown a mechanism that involves a participation of the voltage-gated sodium channels [3]. Similarly to our findings, Oliveira et al. [10] have showed that NST possesses anticonvulsant property in mice and Machado Araújo et al. [21] have demonstrated that NST promotes the blockade of sodium channels from GH3 cells. However, the alteration produced by NST on the time constant of repolarization could also indicate a possible involvement of voltage-gated potassium channels as it was verified by Pisciotta and Prestipino [22] when fenitoine was used. Moreover, an analgesic effect was described for lamotrigine, felbamate, gabapentin, and anticonvulsant drugs in cold allodynia test (chronic constriction injury model) [23].

Based on our results, *N*-salicyloyltryptamine (NST) has antinociceptive activity that may be associated with decreased peripheral nerve excitability. The precise mechanisms underlying the inhibitory effect of NST are not clear. However, its antinociceptive property might involve a voltage-gated sodium channel blocker and nonparticipation of the opioid system. Moreover, these results also support that NST has a therapeutic potential for painful disorders.

Conflict of Interests

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

Acknowledgments

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