Analgesic Activity of 5-HT$_3$ Receptor Antagonists

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The effects of 5-HT$_3$ receptor antagonists tropisetron and condensed derivative of benzimidazole (laboratory code 64B) on nociceptive threshold were examined on models with activation of peripheral and central nociceptive mechanisms. The examined substances demonstrated pronounced analgesic effects in peripheral pain.

Key Words: *5-HT$_3$ receptor antagonists; tropisetron; benzimidazoles; analgesia*

Various types of nociceptors participate in pain perception. A key role in peripheral nociception is played by subtype 3 of serotonin (5-HT$_3$) receptors. These peripheral receptors are located in encapsulated Aδ afferents and in C-fiber afferents, which include peptidergic (substance P) and non-peptidergic subpopulations. Activation of peripheral 5-HT$_3$ receptors depolarized the membrane of nociceptive afferents, which generates the nociceptive signal [12]. In CNS, serotonin facilitates the nociceptive input via spinal 5-HT$_3$ receptors (descending serotoninergic pathways inhibit nociception at the spinal level via stimulation of GABA-ergic inhibitory neurons) [6,10,11].

5-HT$_3$ receptor antagonists used against nausea and vomit resulting from cytostatic chemotherapy demonstrate potent analgesic properties observed on experimental models of peripheral pain [3,5,9,12] and in patients with fibromyalgia and some other peripheral pain syndromes [4,7].

Of special interest among 5-HT$_3$ receptor antagonists are indoles, *e.g.* tropisetron, carbazole derivatives (ondansetron), tropanyl derivatives (MDL72222), and benzimidazoles (granisetron, lerisetron, itasetron, and agent 64B) [1].

Our aim was to study the analgesic properties indole derivative tropisetron and condensed derivative of benzimidazole 64B.

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MATERIALS AND METHODS

Experiments were carried out on random-bred albino male rats (n=162) weighing 180-220 g kept under standard vivarium conditions.

Formalin hyperalgesia [10] was induced by subcutaneous injection of 0.05 ml 1% formalin in the ventrolateral surface of the right hindleg. The nociceptive threshold was measured 2 min postinjection with a mechanical algesimeter. The threshold was determined as the minimum weight producing leg withdrawal reflex. The examined agents and tramadol (Tra-

TABLE 1. Effect of Peroral Tropisetron, Agent 64B, and Tramadol on Compression Nociceptive Threshold in Formalin Hyperalgesia Model ($M\pm m$)

Substance/Group		Nociceptive threshold	
		g	%
Intact control		247.5±15.4	100
Formalin hyperalgesia		153.8±14.3*	62.1
Tropisetron	1 mg/kg	242.0±12.7$^+$	97.8
	10 mg/kg	263.6±31.6$^+$	106.5
Agent 64B	1 mg/kg	257.6±25.4$^+$	104.1
	10 mg/kg	235.2±31.3	95.0
Tramadol	1 mg/kg	214.3±16.1	85.8
	10 mg/kg	301.7±24.4$^+$	121.9

Note. $p\leq0.05$ compared to: *intact controls; $^+$formalin hyperalgesia.

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mal, Polpharma) were administered *per os* 60 min before formalin injection. All substances were used in doses of 1 and 10 mg/kg.

Freund adjuvant hyperalgesia was induced as described previously [8]. Chronic inflammatory hyperalgesia was recorded on day 4 after subcutaneous administration of the adjuvant (0.05 ml mixture of BCG in paraffin oil) in the ventrolateral surface of the right hindleg. The nociceptive threshold was determined as in the previous model 5 min after subcutaneous injection and 60 min after peroral administration of the examined agents and sodium diclofenac used as the reference drug. All substances were administered in doses of 1 and 10 mg/kg.

To produce electric stimulation algesia [10], the tail root was stimulated with noxious rectangular voltage pulses delivered from an ESL-2 generator via bipolar subcutaneous electrodes (10 msec duration, 100 Hz pulse rate, 1-sec stimulation). The nociceptive threshold (tail flick, vocalization, and persistent post-stimulatory vocalization) was measured in volts. Agent 64B and the reference preparation (narcotic analgesic promedol, Moscow Endocrine Plant) were administered *per os* (1 mg/kg) 1 h before stimulation.

The data were processed statistically using Student's *t* test.

TABLE 2. Effect of Tropisetron, Agent 64B, and Sodium Diclofenac on Compression Nociceptive Threshold in Freund's Adjuvant Hyperalgesia Model (*M*±*m*)

Substance/ Group	Mode of administration	Nociceptive threshold	
		g	%
Intact control		247.5±15.4	100
Adjuvant hyperalgesia		154.5±12.5*	62.4
Tropisetron			
1 mg/kg	*per os*	215.5±12.4+	87.1
10 mg/kg		244.5±11.0+	98.8
Agent 64B			
1 mg/kg	Intraplantar	274.4±17.7+	110.9
10 mg/kg		309.0±12.9+	124.8
1 mg/kg	*per os*	225.5±12.4+	91.1
10 mg/kg		266.7±11.0+	107.8
Sodium diclofenac			
1 mg/kg	Intraplantar	121.0±25.4	48.9
10 mg/kg		269.0±13.7+	108.7
1 mg/kg	*per os*	257.0±9.2+	103.8
10 mg/kg		253.3±16.6+	102.3

Note. $p \le 0.05$ compared to: *intact controls; +adjuvant hyperalgesia.

TABLE 3. Effect of Agent 64B and Promedol (1 mg/kg, *per os*) on Nociceptive Threshold during Electrical Stimulation of Tail Root (*M*±*m*)

Substance	Tail flick reflex			Vocalization			Persistent vocalization		
	20 min	60 min	120 min	20 min	60 min	120 min	20 min	60 min	120 min
Control	0.40±0.05			0.60±0.13			1.00±0.26		
Promedol	0.60±0.14	1.3±0.2*	2.4±0.7	0.80±0.19	1.80±0.27*	3.2±0.7*	2.0±0.7	3.1±0.3*	5.0±0.5*
Agent 64B	0.40±0.05	0.50±0.04*	0.50±0.08	0.60±0.14	0.80±0.15	0.80±0.16	0.9±0.2	1.1±0.2*	1.1±0.2*

Note. *$p \le 0.05$ compared to the control.

RESULTS

In intact rats, the nociceptive threshold determined by mechanical compression of the hindlimb was 232-262 g. Injection of formalin induced "nociceptive" behavior (lifting of the affected leg, licking, biting, and shaking). Hyperalgesia was observed approximately 3 min postinjection. Formalin significantly decreased the nociceptive threshold to mechanical compression by 37.9% in comparison with intact rats (Table 1). Administration of all test agents greatly elevated the nociceptive threshold (approximately, to the same degree without significant differences between the substances).

Chronic inflammation produced pronounced hyperalgesia manifested in a 34.6% decrease in the nociceptive threshold (Table 2). The examined agents effectively eliminated this hyperalgesia after peroral or intraplantar administration with the only exception (intraplantar injection of 1 mg/kg sodium diclofenac was ineffective).

The pronounced analgesic properties demonstrated by 5-HT_3 antagonists tropisetron and 64B can be explained by the secondary nature of the pain induced by formalin or Freund's adjuvant (acute and chronic inflammatory agents, respectively). In these cases, the pain is triggered by inflammatory transmitters (*e.g.* serotonin) affecting nociceptive C-fiber terminals. 5-HT_3 receptor blockers produce analgesia by preventing depolarization of C-fibers triggered by 5-HT_3 receptors.

To exclude the involvement of central antinociceptive mechanisms of the test agents, we examined their effects in spinal analgesic test during electrical stimulation of the tail root (Table 3).

The sequence of effects induced by increasing electrical stimulation is explained by hierarchical principle among the structures integrating the nociceptive signals in CNS: the tail withdrawal reflex is originated in the spinal cord, vocalization during stimulation is controlled by the brainstem, and the poststimulation squeal reflects involvement of the thalamus and rhinencephalon [10]. Promedol significantly enhanced the perceptional thresholds of acute spinal pain manifested in all behavioral reactions, the maximum effect was observed 120 min after administration of the drug, when the pain threshold 5-6-fold surpassed the control values. All these CNS subdivisions express opioid receptors, which are the targets for narcotic analgesics, including promedol.

The examined agents had practically no effect on the nociceptive thresholds of electrical stimulation during the entire period of this study, which attests to the absence of central antinociceptive activity in the spinal cord, brainstem, and thalamus. The absence of effects of 5-HT_3-antagonists on the nociceptive traffic in CNS is corroborated by published data on the profile of antinociceptive activity of 5-HT_3-receptor blockers.

Our data attest to pronounced analgesic activity of the examined 5-HT_3-antagonists, tropisetron and agent 64B, which was observed on the experimental models of peripheral pain. Analgesic potency of these chemicals was no less than that of tramadol, and in low doses, it was even more potent than sodium diclofenac.

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