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PENTOXIFYLLINE MODIFIES CENTRAL AND PERIPHERAL VAGAL MECHANISM IN ACUTE AND CHRONIC PAIN MODELS

Abstract: *Pentoxifylline modifies central and peripheral vagal mechanism in acute and chronic pain models.*

The inflammatory process gives the way to hyperalgesia that is documented by the animal experimental studies. Pentoxifylline (PTX) has strong antiinflammatory effects, decreases TNF-alpha and other proinflammatory cytokines production. Therefore, the aim of present investigation was to evaluate the effectiveness of PTX in nociception processes, especially in aspects of vagal activity, in experimental pain models: visceral pain (VP), neuropathy (CCI) and neurogenic inflammation (NI). In VP and CCI models we observed significant increase in the pain threshold after blocking proinflammatory cytokines whereas in NI there was no such effect. In our studies we also observed the increase of vagal afferents activity in VP and CCI, on the contrary to NI model. In summary, our study demonstrates that preemptive inhibition of proinflammatory cytokine synthesis by treatment with PTX is useful in antagonizing hyperalgesia in inflammatory pain. Pentoxifylline reduces central and peripheral sensitization processes depend on the vagal component in both acute and chronic pain models but in a different manner and mechanisms. Our results establish the participation of inflammatory and vagal component in nociception. The modulation of the vagal system offers the new possibilities of the pain treatment in patients resistant to the classical analgesic therapy.

Key words: pentoxifylline, visceral pain, neuropathy, neurogenic inflammation, vagus nerve

INTRODUCTION

There is increasing evidence showing that sensitization processes are not only controlled by spinal afferent fibers but it is also related to vagal afferent fibers [1–4]. Hypersensitivity in response to mechanical and chemical stimuli results from the sensitization of the primary afferent neurons (peripheral hyperalgesia)

and the nociceptive pathways in the central nervous system (CNS) (central hyperalgesia) [5, 6]. The vagus nerve, the largest sensory nerve in the body, and its involvement in inflammatory response, can be a target for anti-pain therapy lately, due to growing evidence that indicates its contribution in nociception.

Vagotomy studies demonstrate that vagal afferent integrity is essential to the efficacy of different analgesic treatments like morphine [7]. Furthermore, subdiaphragmatic vagotomy decreases the threshold for paw withdrawal in rats [8], increases sensitivity to noxious lesions [9] and enhances hyperalgesia induced by the potent inflammatory mediator bradykinin [10, 11]. However, vagotomy also prevents the establishment of kainic acid-induced hyperalgesia in mice [12] and reduces nociception in the formalin test in male rats [13]. On the other hand an anti-nociceptive action of VNS is reported in numerous studies which describe for instance inhibition of the nociceptive reflex [14, 15] or latency increase of the tail-flick response to heat in rats [16]. However, pronociceptive effects have also been reported in the tail-flick test in rats [17]. These discrepancies can be explained by differences in stimulation parameters : low intensity stimulations of vagal afferents facilitate, while high intensity stimulations inhibit nociception [7]. According to Ren et al., the analgesic effect of VNS seems to depend on a critical stimulation intensity that activates C-fibers [18].

Pentoxifylline (PTX), a nonspecific phosphodiesterase inhibitor, has several actions that improve blood rheology and tissue perfusion and it is used clinically as a treatment for intermittent claudication [19]. Several laboratory investigations, however, employ the drug for its anticytokine effects. Previously several reports demonstrate that PTX administration virtually eliminates the up-regulation of cytokines in various pain models [20–26]. Its influence on inflammatory component of sensitization processes is well known, but on the vagal dependent mechanisms, remain unclear [27]. Thus the aim of present investigation was to evaluate the effectiveness of PTX in peripheral and central sensitization processes, especially in aspects of vagal activity, in experimental pain models; visceral pain (VP), neuropathy (CCI) and neurogenic inflammation (NI).

MATERIALS AND METHODS

Experimental procedures were performed on 130 Wistar rats in Pathophysiology Department of the Collegium Medicum, Jagiellonian University in Kraków. All the procedures were conducted with the approval of the I Local Ethical Committee for the Animal Studies in Kraków.

In this study we analyzed modification of vagus nerve activity (electrical activity) and c-Fos expression in vagal nodose ganglion (NG) after PTX administration. To estimate the intensity of pain symptoms manifestations we provided the behavioral tests adequate to the pain model: abdominal constriction test (WT) in VP, von Frey test to assess mechanical allodynia and Hargreaves test to assess thermal hyperalgesia in CCI and NI.

Pain models

Visceral Pain (VP). There was used accepted animal model of the viscerosomatic pain [28, 29]. By injecting i.p. 0,5% acetic acid (10 ml/kg) there was induced chemical peritonitis.

Neuropathic pain (CCI). In the experiment there was used Bennet and Xie model of chronic constriction injury (CCI) [30]. The behavioral tests were performed before the surgical procedure (day 0) and then on 14th day after the procedure. In our experiments the control group was the one, in which there was conducted the surgical procedure but without injuring the nerve itself (sham), additionally the healthy leg (contralateral, contra) — in every subgroup served as a control. These was evaluated and compared to the group with “pure” neuropathic pain (CCI) as the behavioral response of the injured leg (ipsilateral — ipsi).

Neurogenic Inflammation (NI). Approved Gilchrist’s model was used for induction of NI. Mechanical and thermal stimuli were administered 3 mm proximally to capsaicin injection place (highest pain response zone) [31].

Behavioral tests

Behavioral response in acute visceral pain — Writhing Test (WT). Intraperitoneal administration of acetic acid causes peritonitis and behavioral manifestation of nociception. The intensity of nociception was measured by counting of the rat’s abdominal and hind limb muscles contractions (WT) in a period of 30 minutes after acetic acid administration.

Von Frey’s test — test of mechanical allodynia. The von Frey’s test is used to evaluation of the behavioral response to mechanical nociceptive stimuli [32, 33]. The experimental procedures were conducted by using automatic von Frey’s filament (Dynamic Plantar Aesthesiometer — Ugo Basile, Italy).

Hargreaves’ Test — test of thermal hyperalgesia. The Hargreaves’ test is used to evaluation of the sensitivity to the thermal nociceptive stimuli [34]. To the tests there was used Plantar Test apparatus (Ugo Basile, Italy).

Electrical activity of the left vagus nerve

Under general anesthesia the left vagus nerve was exposed on the neck and recorded because it contains more afferent fibers [35]. Furthermore in the trials conducted in the Pathophysiology Department there weren't observed any differences between the left and right vagus nerve activity [36]. The nerve trunk was dissected transversely. On the distal part cuff electrode was placed. The signal was amplified and converted by using the standard set (PowerLab/8SP ADInstruments Australia and AC/DC Differential Amplifier A-M Systems USA). The records were archived and then worked up by using packet of software Chart 5 Pro ver. 5.4.2 extended with Spike Histogram v.1.2 (ADInstruments, Australia), compatible with Windows XP system. All the results were given as the mean \pm standard deviation and statistically worked out. For the significant accepted p values were $p < 0,05$.

c-Fos expression in nodose ganglion (NG)

After Vetbutal overdosing inferior vagal ganglions (NG, nodose ganglions) were bilaterally prepared and sampled to histochemical evaluations. The preparations were fixed and then frozen. Frozen preparation was sliced by using cryostat and subsequently submitted to the immunohistochemical staining. The specimens were incubated with rabbit c-Fos antibody (K-25; Sc-253) (Santa Cruz Biotechnology). After 3 times repeated PBS washing the specimens were incubated with second degree biotinylated goat anti-rabbit antibody (Jackson ImmunoResearch, West Grave, PA). Afterwards the specimens were incubated with streptavidin — Cy3 complex (Jackson ImmunoResearch, West Grave, PA). Then the specimens were embedded in fluorescent specimen's medium (DAKOCytomation, Denmark). Negative controls were conducted without the first antibody. The specimens were analyzed under the Zeiss Axsioscop fluorescent microscope.

PTX pre-emptive analgesy

To the rats in the proper groups there was pre — emptively administered i.p. PTX in a dosage 10 mg/kg b.m. In the VP and NI groups PTX was given 15 minutes before conducting the behavioral tests. In the CCI group PTX was administered every 12 hours during the experimental duration time — first dosage the animals received 6 hours prior to the surgery. The aim was to limit the inflammatory response in the evaluated groups.

Statistical analysis

The calculations were performed with using STATISTICA 8.0 for Windows packet (StatSoft Inc., Tulsa, Oklahoma, USA). The results were given in the tabular and graphic form with using Microsoft Office Excel 2007 software. Conformity of the quantitative variables distribution with the normal distribution was evaluated with using Shapiro–Wilk test. The results obtained from the analysis were given as the arithmetical mean \pm standard deviation. As the statistically significant results there were accepted those with the difference on the level $p < 0,05$. Graphs were made as deviation from control group (“pure” pain model).

RESULTS

Visceral pain (VP). In model of VP we monitored the direct participation of the vagus nerve in mechanisms of visceral nociception which dominated over classic spinal pain pathways. PTX administration before experiment in visceral nociception decreased the pain response. Intra-peritoneal acetic acid administration increased both the vagal afferent discharges frequency and c-Fos expression in NG whereas pre-PTX administration suppressed this effect (Fig. 1, Tab. 1).

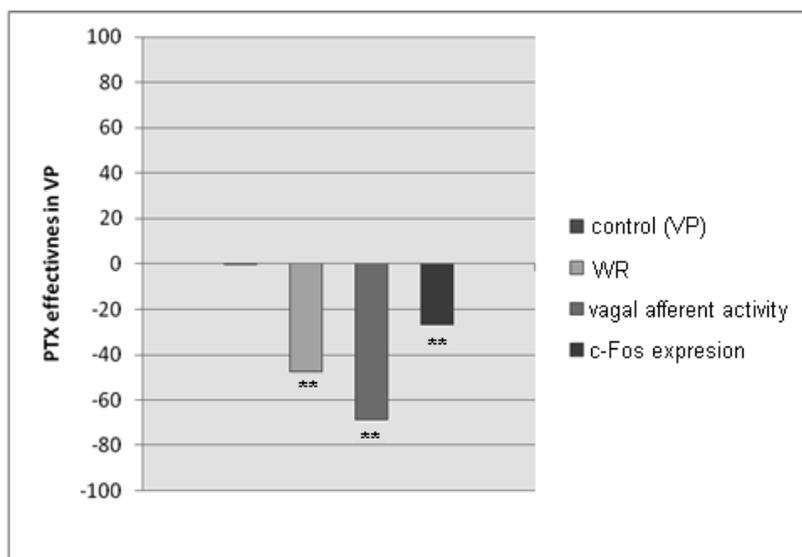


Fig. 1. Pain response (WR) and vagal activity after PTX administration in visceral pain. Results are shown as deviation from VP group (0%) (** — $p < 0.01$)

Table 1

Pain response (WR) and vagal activity after PTX administration in visceral pain
 WR — number of writhes/30 min, Vagal Afferent Activity — frequency of impulses (Hz),
 c-Fos Expression — number of c-Fos positive cells/slide of NG

	WR	Vagal Afferent Activity	c-Fos Expression
control	42.3 ± 2.1	1.78 ± 0.34	18 ± 1.5
VP+PTX	22.2 ± 1.9	0.56 ± 0.17	13.2 ± 1.3

Neuropathic pain (CCI). The vagus nerve plays an important role in sensitization through the development of chronic pain (CCI). In model of chronic pain (CCI) the vagus nerve is participating indirectly in both of peripheral and central sensitisation. The pain threshold significantly increased after PTX. It allows to assume, that on the development of mechanical allodynia and of thermal hyperalgesia contribution of the vagus nerve is essential. Vagal afferents activity and c-fos expression in NG neurons in the CCI group increased. In the PTX group the vagus nerve activity and the c-Fos expression were lower in comparison to CCI (Fig. 2, Tab. 2), it demonstrate central modification of vagal components.

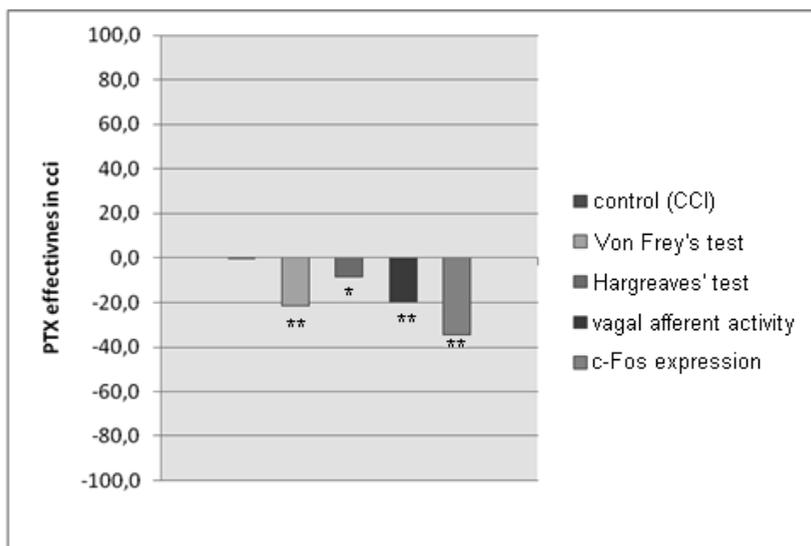


Fig. 2. Behavioral Tests and vagal activity after PTX administration in CCI model. Results are shown as deviation from "pure" CCI group (0) (* — < 0.05, ** — p < 0.01)

Table 2

Behavioral Tests and vagal activity after PTX administration in CCI model
 Von Frey's Test — filament pressure (g), Hargreave's Test — time of paw withdrawal (s),
 Vagal Afferent Activity — frequency of impulses (Hz), c-Fos Expression — number of c-Fos
 positive cells/slide of NG

	Von Frey's Test	Hargreave's Test	Vagal Afferent Activity	c-Fos Expression
control (CCI)	15.4 ± 1.4	6.5 ± 0.9	0.31 ± 0.05	20.9 ± 1.4
CCI+PTX	18.7 ± 1.7	7.1 ± 0.8	0.25 ± 0.03	15.8 ± 1.2

Neurogenic inflammation (NI). We observed attenuation of vagal afferent activity in the left vagus nerve and absence of c-Fos expression in NG in the NI group. PTX didn't influence the activity of the vagus nerve (Fig. 3, Tab. 3). These results support hypothesis of blockade of the central vagal centers in order to facilitate the nociceptive information transmitted by spinal pathways.

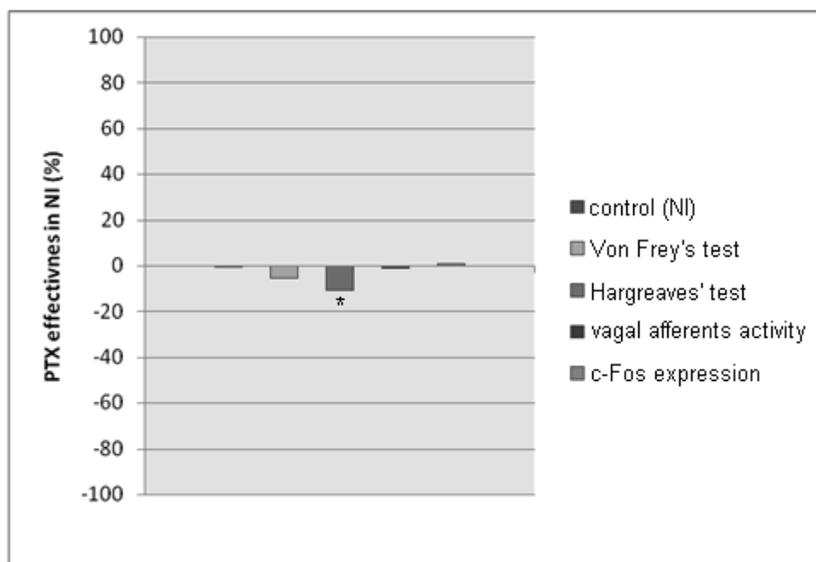


Fig. 3. Behavioral Tests and vagal activity after PTX administration in NI model. Results are shown as deviation from "pure" NI group (0) (* — $p < 0.05$)

Table 3

Behavioral Tests and vagal activity after PTX administration in NI model
 Von Frey's Test — filament pressure (g), Hargreave's Test — time of paw withdrawal (s),
 Vagal Afferent Activity — frequency of impulses (Hz), c-Fos Expression — number of c-Fos
 positive cells/slide of NG

	Von Frey's Test	Hargreave's Test	Vagal Afferents Activity	c-Fos Expression
control	18.2 ± 2	7.6 ± 1	0.06 ± 0.01	5.1 ± 1
NI+PTX	19.06 ± 1.25	8.4 ± 1.1	0.057 ± 0.01	5.4 ± 1.7

DISCUSSION

PTX has strong anti-inflammatory effects, decreases TNF-alpha and other proinflammatory cytokines (Il-1, Il-6, Il-8) production [37, 38]. In VP and CCI models we observed significant increase in the pain threshold after blocking proinflammatory cytokines (PTX) whereas in NI there was no such effect [39]. Development of the local inflammatory process activates the cells of the immune system, which release numerous inflammatory mediators, such as cytokines, leukotrienes, prostaglandins, proteases, kinins, histamine. These mediators activate the sensory neurons or/and change their sensitivity [40–43]. The inflammatory process gives the way to hyperalgesia that is documented by the animal experimental studies [44, 45]. In our studies we observed the increased electrical activity of the left vagus nerve in VP and CCI, on the contrary to NI model. Vagal afferent fibers, due to the expression of the proinflammatory cytokines receptors, have a special place in the communication between immune system and CNS [46, 47]. Most of the authors describes the vagus nerve as responsible mostly for leading the physiological stimuli [48], however there are several data confirming its role in pathophysiology of the nociceptive processes [4, 49, 50]. Pain stimulation after tissue damage and inflammation modifies nervous system response in important processes of peripheral and central sensitization. Vagus nerve plays a crucial role in the development of the autonomic anti-inflammatory reflexes [51, 52]. This confirms the Watkins *et al.* data, stating that post-inflammatory hyperalgesia depends on the vagal afferent pathways integrity [53]. PTX was more effective in reduce of vagal afferents discharge in VP than in CCI model and it wasn't effective in NI model. We also observed that PTX administration significantly reduced c-Fos expression in nodose ganglion in VP, and even stronger in CCI model. In CCI model c-Fos expression in Dorsal Root Ganglia (DRG), spinal cord [54, 55], and in NTS [56, 57] increased. Complete dissection of the sciatic nerve also increased c-Fos expression in spinal cord [58] that with reference to our studies may prove the central sensitization, which may be mediated via vagal pathway. However

in antigen-induced arthritis (AIA) the c-Fos expression decreased in spinal cord [59]. According to Zhao *et al.* pain response to mechanical stimuli and to increased temperature in neuropathy doesn't depend on the sympathetic nervous system [60]. There exists no marked evidence concerning the vagal participation in the acute peripheral pain models. Data obtained in our studies show that vagal afferent endings plays the crucial role in the chemical peritonitis model (VP). We observed increased vagal afferents activity and c-Fos expression in NG. Our finding is consistent with the others studies, reporting increase of gastric vagal afferents discharge and c-Fos expression in NTS induced by the different types of noxious stimuli [61, 62]. Holzer *et al.* show that exposure of the rat gastric mucosa to noxious stimuli is signaled to the brainstem, but not spinal cord. This observation is complemented by the finding that the visceromotor response to gastric acid challenge is suppressed by vagotomy, but not splanchnectomy [61, 63]. In other studies after intraperitoneal injection of IL-1 β and LPS increased c-Fos expression in NTS and NG also confirms our results [64, 65]. The involvement of the central modulation of spinal antinociceptive systems with the vagus nerve was observed in the rectum stretching model [66]. The participation of the vagal component in the chronic pain doesn't limit itself only to the central nociception modulation. In the CCI model we observed complex, coming probably from the existence of "neuronal plasticity", vagal participation in the modulation, both peripheral and central sensitization [5, 6]. These observations suggest that the vagus nerve may influence nociceptive information on many levels of its transmission, being in a close correlation with the immune and endocrine system especially at CNS level. In NI model we observed also the slight influence of the pre-emptively administered PTX on thermal hyperalgesia that could be a result of the acute character of the study and a lack of proper time for complete development of central sensitization [67]. Similar conclusions are coming from Obara *et al.* studies, where there was observed in CCI bigger influence of the inflammatory component on the pain threshold increase than in NI [68]. This may confirm the crucial role of the classic, nociceptive pathway in this model. Our results establish the participation of inflammatory and vagal component in nociception what can constitute the base for using this tracks in both acute and chronic pain complexes treatment. Anti-inflammatory substances (like PTX), by decreasing spinal and vagal activity, may modify peripheral and central sensitization. The vagus nerve is directly involved in the visceral nociceptive mechanisms, showing the predominance over the classical, spinal nociceptive pathways. Its activity depends on the inflammatory component — so, it shows also antinociceptive action, dependent on the efferent pathways of the inflammatory reflex. In the neuropathic pain model vagus nerve is indirectly involved, playing its role in the peripheral and central sensitization mechanisms. The vagus nerve may modulate the higher CNS centers that are taking part in the nociception (classical

antinociceptive systems, endocrine system (HPA axis)). There is observed the central modulation of the vagal system through the spinal component that is playing the crucial role in the peripheral nociception. Pre-emptive blockage of the proinflammatory cytokines diminishes the development of the neuropathic signs. Suppression of pro-inflammatory mediators synthesis in NI attenuates pain response not significantly what can result from the used type of acute pain model and insufficient time needed for the full sensitization development.

In summary, our study demonstrates that preemptive inhibition of pro-inflammatory cytokine synthesis by treatment with PTX is useful in antagonizing hyperalgesia in inflammatory pain. Pentoxifylline reduces central and peripheral sensitization processes depend on the vagal component in both acute and chronic pain models but in a different manner and mechanisms. Our results establish the participation of inflammatory and vagal component in nociception. The modulation of the vagal system offers the new possibilities of the pain treatment in patients resistant to the classical analgesic therapy.

CONFLICT OF INTERESTS STATEMENT

None declared.

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